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CONCENTRATION OF BRAZILIAN TOBACCO STREAK VIRUS IN INFECTED PLANTS [1]

A. S. Costa [2]

A certain degree of recovery seems to be of rather wide occurrence among plant virus diseases. Acute symptoms are shown in the initial stages of infection, followed by chronic symptoms of a milder degree. For some diseases the difference in severity between symptoms of the acute and those of the chronic stage is very striking and consistent, apparently indicating that some sort of mechanism has become operative that is beneficial to the plant.

One of the explanations for the milder symptoms of the chronic or recovery stage is that this stage results from a process that somehow limits virus increase in tissues that subsequently will show recovery. As a result, the virus concentration in cells of such recovered tissues usually would be lower than in cells of tissues with acute symptoms.

Price (6) presented extensive data showing that plants of tobacco (*Nicotiana tabacum* L.) that had recovered from ringspot had a lower concentration of tobacco ringspot virus (*Annulus tabaci* Holmes) in recovered leaves than was present in leaves that showed necrotic symptoms. Price's findings were confirmed by Stanley (7), who isolated greater quantities of tobacco ringspot virus from leaves showing acute symptoms than from recovered leaves. More recently, Bennett (1) reported that in plants of various species recovered from the disease caused by dodder latent mosaic virus (*Marmor secretum* Bennett), tissues that showed severe symptoms had a higher virus concentration than recovered tissues. Bennett further demonstrated the correlation between virus concentration and severity of symptoms by introducing a second virus into the recovered plant; this induced the appearance of dodder latent mosaic symptoms, but the concentration of this virus also was increased.

In recent studies on the concentration of tobacco streak virus (*Annulus orae* Holmes) in necrotic and recovered tissues of tobacco plants, Fulton (5) concluded that the production of new, symptomless leaves was not due to reduction in amount, or in virulence, of the virus present in them. These results were confirmed by Diachun and Valleau (4), who obtained more local lesions with inoculum from young tobacco leaves in the chronic stage than with inoculum from acutely necrotic leaves.

A virus disease in tobacco fields in the State of São Paulo, Brazil (3), offers many points of similarity to American tobacco streak, and is considered to be caused by a strain of this streak virus.[3] Tobacco plants infected by the Brazilian streak virus also show an acute and a chronic, or recovery, stage (Fig. 1, A). Hence it seemed of interest to test whether recovery in the Brazilian tobacco disease was, or was not, accompanied by a decrease in virus concentration in cells of recovered tissues.

MATERIAL AND METHODS.—Turkish tobacco plants used in the tests were grown in 6-in. pots filled with manured soil. The Brazilian tobacco streak virus used was a field collection made years ago. Although not a single-lesion strain, this virus collection has given very uniform results in past tests. The estimates of virus concentration in samples were based on number of lesions obtained by inoculation of *Beta patellaris* Moq. (2). Leaves of this plant furnish local lesions good for counts when inoculated mechanically with the Brazilian tobacco streak virus (Fig. 1, B). The lesions become visible usually 2 days after inoculation, and can be counted after 4 days. In order to obtain good test plants, seedlings of *B. patellaris* were planted singly in 8-in. pots filled with heavily manured soil. The plants were permitted to develop only one stem, and were suckered when necessary. The same plant could be used two or three times if the stem was cut back and a new shoot allowed to grow. Care in handling the test plants was necessary because the leaves and petioles broke easily.

Tests for virus concentration were made on disk samples (5) taken with a sterile cork borer 7/16 in. in diameter. If two samples to be compared were taken from leaves of different ages, disks from healthy leaves of comparable ages were added to the inocula, to minimize possible differences in the amount of inhibitor formed when the tissues were ground (5). Disks from younger healthy leaves were then mixed in equal numbers, before grinding, with those from older diseased leaves, and vice-versa. Disks from healthy leaves were not added to the inocula when these were taken from leaves of about the same physiological age.

Disks were ground in a mortar with enough 0.01M sodium sulphite added for the desired dilution. Carborundum powder (600-mesh) was sprinkled on the leaves before inoculation, in all tests.

Pairs of samples were compared on opposite half-leaves. The position of each pair reversed so that the samples would occupy the same number of left and right half-leaves. With one concentration of various samples, all possible combinations were paired. When more than one concentration of two samples were compared, the pairing was done with the same con-

[1] Accepted for publication December 4, 1951.

[2] Pathologist, Instituto Agronômico de Campinas, Brazil.

The writer in indebted to Drs. W. D. Valleau, Stephen Diachun, and C. W. Bennett for criticisms made on a preliminary draft of this paper.

[3] Unpublished paper.

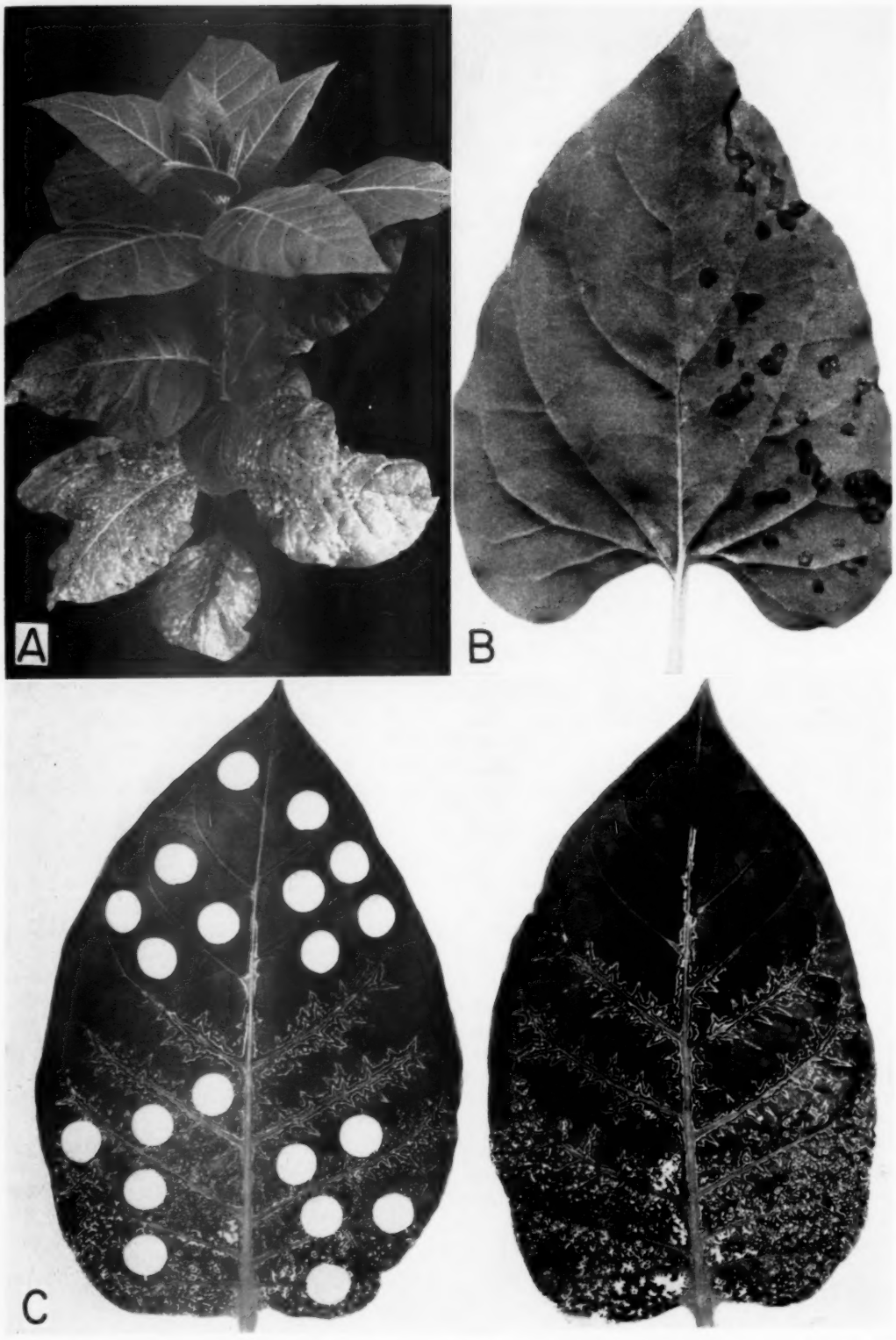

FIG. 1. A. Turkish tobacco plant in the recovery stage, showing streak in the lower leaves and almost symptomless upper leaves. B. Necrotic lesions caused by the Brazilian tobacco streak virus in leaves of *Beta patellaris*, about 8 days after inoculation. C. Necrotic and non-necrotic areas in leaves showing systemic necrosis.

centration of the samples. All pairs of treatments were distributed on the plants at random.

VIRUS ACTIVITY.—*Time of sampling.*—Preliminary attempts to determine the concentration of Brazilian tobacco streak virus in leaves of tobacco plants showing various types of symptoms soon indicated that the manner of sampling had a great bearing on the number of lesions obtained. For instance, virus activity in inoculated tobacco leaves was usually high when samples were taken a few days after inoculation, but low when they were taken a long time after. Similar observations on samples from leaves showing systemic necrosis and from recovered leaves indicated that virus activity in the samples varied significantly, and that in order to compare samples from different parts of the same plant or from different plants it became important to know the approximate time when virus activity in the tissues was at the peak.

In inoculated leaves.—The five lower leaves of five Turkish tobacco plants were inoculated with a 1:5 dilution of the virus, and disks from these leaves were taken at intervals and tested for virus activity; results are in Table 1. Activity was low on the 2nd day after inoculation, and relatively high on the 6th day; a decrease beginning on the 16th day was very definite on the 27th day.

TABLE 1.—*Concentration of Brazilian tobacco streak virus in samples taken from the same inoculated tobacco leaves at different intervals*

Plant number, and day (after inoculation) of sampling	Average[a] number of lesions per halfleaf of *Beta patellaris* when inoculum was taken from leaf number[b]				
	1	2	3	4	5
1: 2	28.8	57.0	21.3	60.8	21.8
6	89.1	87.1	157.4	172.4	150.9
16	86.9	116.0	123.5	128.8	52.5
27	4.4	3.5	6.3	2.4	2.1
2: 2	1.8	11.1	3.6	1.6	4.0
6	125.9	157.8	131.8	131.9	147.6
16	101.6	120.0	113.9	118.4	57.6
27	0.9	4.5	4.8	5.8	5.5
3: 2	10.4	0.3	0.9	3.0	0.0
6	67.0	109.3	121.9	119.8	138.8
16	30.5	17.4	23.6	18.4	28.5
27	—	4.8	4.8	2.1	1.4
4: 2	4.4	28.0	25.6	12.9	16.0
6	88.4	101.0	95.1	101.4	130.1
16	17.3	57.4	54.4	31.4	46.4
27	—	—	—	—	—
5: 2	5.3	15.3	28.6	38.4	46.1
6	67.6	101.3	92.3	129.6	105.4
16	40.6	25.0	41.4	58.3	68.3
27	—	—	13.0	14.3	17.5

[a] Average number of lesions based on 10 half-leaves.
[b] Leaf No. 1 was the lowest inoculated; 2, 3, 4, and 5 the leaves immediately above.

In tests 1 to 6 from Table 3, samples from inoculated leaves of plants in the recovery stage averaged 2.6 lesions per half-leaf of *Beta patellaris*. In tests 7 to 10, in which the samples were taken from inoculated leaves of plants that had just shown incipient, systemic necrosis, lesions per half-leaf averaged 89.9.

In leaves with systemic necrosis.—Virus activity in leaves with systemic necrosis also increases, reaches a peak, and then decreases as the leaves become older. In one test, disks were taken from the upper leaves of a tobacco plant as soon as virus symptoms could be seen, and ground with enough 0.01M sodium sulphite to give a 1:10 dilution; this inoculum caused an average of 8.7 lesions per half-leaf of *B. patellaris*. Samples from the same leaves, taken 2 days later, treated and used as inoculum in the same manner, induced an average of 188.8 lesions per half-leaf, and samples tested 15 days later caused an average of 36.2 lesions. In another test samples from leaves of two plants showing incipient systemic necrosis gave, respectively, 214.4 and 203.8 lesions per half-leaf; tested 8 days later, 133.3 and 155.2 lesions; and 23 days after the first test, 14.8 and 19.1 lesions. Results in Table 3 also show that the number of lesions obtained with samples from leaves showing incipient systemic necrosis was much higher than from leaves with the same type of symptom but from plants in the recovery stage.

In recovered leaves.—Virus activity decreases also in recovered leaves, but not always so strikingly as in leaves that showed either local or systemic necrosis. Disks from recovered tobacco leaves at various levels on the plant usually show an activity gradient decreasing from the top. A similar observation was made by Diachun and Valleau (4) for plants of *Nicotiana rustica* L. infected with the tobacco streak virus.

Disks from the same recovered leaves were tested at intervals and showed a consistent decrease in virus activity (Table 2). Since virus activity was determined in samples taken at intervals, it is necessary to consider that because of cell growth the disks taken on a later date had a smaller number of cells than disks of similar diameter obtained previously from the same leaf.

Comparison of inoculated leaves, leaves with systemic necrosis, and recovered leaves.—In five preliminary tests the virus concentration in disks from recovered leaves was compared with that in leaves of the same plant showing systemic necrosis, and with samples from leaves of another plant in which systemic necrosis was incipient. The samples were ground in presence of enough 0.01M sodium sulphite to give a 1:10 dilution, and each inoculum was tested on 12 half-leaves. In four of these tests the samples from leaves with incipient necrosis caused a greater number of lesions than did those from recovered leaves. In one test the result was reversed. The number of lesions produced with inoculum prepared from leaves that had shown systemic necrosis, of plants in the recovered stage, was very low. The average number of lesions per half-leaf in the five tests was: recovered leaves, 49.9; systemic necrosis (old) from same plant, 1.4; systemic necrosis (incipient) from another plant, 59.2.

TABLE 2.—*Concentration of Brazilian tobacco streak virus in samples taken from the same recovered leaves at different intervals*

Plant number, and interval (in days) between sample testing		Average[a] number of lesions per half-leaf of *Beta patellaris* when inoculum was taken from recovered leaf number[b]				
		1	2	3	4	5
1:	—	142.3	282.5	—	—	—
	13	4.3	24.8	103.7	84.7	—
	12	7.4	29.1	82.5	68.8	105.6
	22	16.1	27.4	4.3	0.5	2.5
2:	—	114.5	202.5	—	—	—
	13	3.0	49.2	102.0	197.0	—
	12	5.5	1.9	20.0	20.0	49.3
	22	12.4	41.6	14.6	22.3	54.3
3:	—	28.0	80.8	—	—	—
	13	6.2	25.0	88.0	167.2	—
	12	2.4	0.6	34.6	89.8	192.6
	22	23.8	2.1	4.8	2.9	10.1
4:	—	27.5	99.0	—	—	—
	13	5.0	42.5	95.0	160.3	—
	12	6.0	26.1	49.6	129.8	264.0
	22	—	11.3	1.3	5.1	6.1
5:	—	26.5	81.2	—	—	—
	13	16.2	47.8	75.5	83.5	—
	12	18.8	25.5	95.3	101.4	172.9
	22	16.3	22.0	11.4	5.6	3.5
6:	—	67.3	100.0	—	—	—
	13	25.0	48.8	55.7	130.8	—
	12	23.0	23.3	55.9	243.9	179.4
	22	5.8	2.1	1.4	5.0	76.1

[a] Average number of lesions based on eight half-leaves.
[b] Recovered leaves 1 to 5 from same plant. Leaf 2 was fifth above 1; leaf 3, fifth above 2, and so on.

In another series of ten tests the same three types of inoculum and samples from necrotic areas of the inoculated leaves were compared, at 1:10 dilution, on six half-leaves; results are in Table 3. In tests 1 to 6, the samples from inoculated leaves were taken from the same plants which supplied the disks with systemic necrosis (old), and the disks of recovered tissues. The disk samples of incipient systemic necrosis were taken from other plants. In tests 7 to 10, the samples from the inoculated leaves and from leaves with systemic necrosis (incipient) were from the same plants, and the samples from leaves with systemic necrosis (old) and from recovered leaves were from other plants. The number of lesions induced by the inocula from leaves with incipient systemic necrosis was generally higher than was obtained with inocula from recovered leaves, although the difference was not very significant. Samples from leaves showing systemic necrosis, from plants that already had many recovered leaves, induced very few lesions. The number of lesions obtained with samples from inoculated leaves, taken when the plants had recovered, was also low. Samples from recently inoculated leaves gave a high number of lesions.

TABLE 3.—*Relative concentration of Brazilian tobacco streak virus in tissues of infected Turkish tobacco plants*

Test No.	Average[a] number of lesions per half-leaf of *Beta patellaris* obtained with inoculum from leaves:			
	Previously inoculated[b]	With systemic necrosis (recent)	With systemic necrosis (old)	Recovered (chronic stage)
1	0.5	125.7	0.4	105.0
2	0.8	82.7	3.5	74.7
3	0.7	82.8	13.3	81.5
4	1.3	110.5	14.8	76.8
5	9.6	93.3	16.3	102.8
6	2.8	87.5	9.7	65.0
7	77.8	113.8	0.0	108.3
8	97.0	136.2	0.3	155.6
9	96.2	188.8	4.0	133.3
10	88.5	135.5	1.2	61.7

[a] Average number of lesions based on six half-leaves.
[b] Samples used in tests 1 to 6 were taken from recovered plants. In tests 7 to 10 the samples were taken from the same plants that had incipient systemic necrosis.

In a third test series, the virus concentration in leaves showing incipient systemic necrosis was compared at three dilution levels with inoculum taken from young recovered leaves of another plant. Dilutions were prepared by a method similar to that used by Fulton (5): disks taken from leaves of the designated type were randomized, and for each type of inoculum the amount of 0.01M sodium sulphite necessary to obtain the desired dilution was added to the same number of disks. Each dilution was prepared just before it was inoculated, to avoid inactivation as much as possible. The results are in Table 4. In six of nine tests, virus concentration in disks from tissues showing incipient systemic necrosis was higher than in disks from recovered leaves. This result was reversed in one test, and was indefinite in two others.

TABLE 4.—*Relative concentration of Brazilian tobacco streak virus in necrotic and recovered tissues as compared at three dilution levels*

Test No.	Average[a] number of lesions per half-leaf of *Beta patellaris* obtained with inoculum[b] at the concentrations indicated:					
	5−1		5−2		5−3	
	SN	RL	SN	RL	SN	RL
1	172	18	124	120	14	24
2	144	36	71	65	10	9
3	214	205	138	176	44	39
4	194	132	133	32	3	3
5	219	192	189	161	39	7
6	293	134	186	49	60	4
7	128	180	70	91	25	38
8	261	170	74	53	32	12
9	324	240	228	124	82	35

[a] Average number of lesions based on six half-leaves.
[b] Source: SN, systemic necrosis; RL, recovered leaves.

VIRUS DISTRIBUTION: *In necrotic and non-necrotic areas of leaves with systemic necrosis.*—Leaves from tobacco plants showing systemic necrosis often have large areas near the tip or between the secondary

veins, and small areas between necrotic lesions, which do not show symptoms (Fig. 1, C). To determine whether virus was present in the non-necrotic areas of these leaves, disks were taken separately from the necrotic and from the non-necrotic areas of individual leaves of eight different plants. Disks from necrotic areas had virus, but in only one case was a little virus recovered from a non-necrotic area; this, however, might have been the result of taking a disk from an area too close to a lesion. The great variation in number of lesions obtained with samples from necrotic areas (from 9.5 to 189.5 per half-leaf) is to be attributed to the fact that the samples were taken from leaves that had shown systemic necrosis at different times.

INOCULATION OF NON-NECROTIC AREAS OF LEAVES SHOWING SYSTEMIC NECROSIS.—Additional evidence that non-necrotic areas in leaves with systemic necrosis are not invaded by the streak virus was obtained by inoculation. Non-necrotic areas of leaves with systemic necrosis of four different plants were inoculated with a 1:10 dilution of the virus. At the same time, leaves of comparable age on healthy plants, and on plants at the recovered stage, were inoculated. After 4 to 5 days the inoculated, non-necrotic areas of leaves with systemic necrosis showed the same type of local lesion that appeared in leaves of the inoculated controls. The leaves of recovered plants inoculated simultaneously did not develop lesions.

DISCUSSION.—Studies on the Brazilian tobacco streak disease indicated that virus distribution in leaves showing systemic necrosis is not complete as in recovered leaves, and that some green areas between the necrotic tissues may be virus-free. Valleau (8) reported that leaves of tobacco plants in the ringspot or acute stage were not completely invaded by the tobacco ringspot virus, and that the normal blade tissues between necrotic spots had no virus.

Data from determinations of virus concentration in samples taken at intervals from the same individual leaves indicated that a decrease in activity of the Brazilian tobacco streak virus in the tissues occurs as the leaves age, especially in those that showed necrosis. Since the determinations were made on disk samples, the decrease in virus concentration in the cells was of a lesser degree than the results indicated, because the samples tested later had a smaller number of cells per disk, as a result of leaf expansion during the interval between sample collections.

The fact that virus activity varies in samples taken from the same leaves at different times requires that comparative measurement of virus concentration in leaves showing different types of symptoms should be made on samples in which virus activity is at the peak. Since the highest concentration in leaves showing systemic necrosis is apparently reached before the plants have reached the recovery stage, the comparison of virus activity in these two types of tissues is based necessarily on samples from different plants. Furthermore, even when the samples are collected from the leaves when concentration is at peak, the results obtained permit a comparison of concentration per area of leaf tissue and not in cells or a group of cells. A technique that would permit sampling the protoplasm of single cells, or of a small group of cells, would be desirable to compare the virus concentration in cells undergoing necrosis and in cells of recovered or chronic tissues. For Brazilian tobacco streak virus or other viruses that are easily inactivated, it might be difficult to carry on such tests.

Tests with the Brazilian tobacco streak virus have shown that virus activity in areas of leaves which presented early stages of systemic necrosis was slightly higher than in comparable areas of recovered leaves, although the difference was not significant. Since the comparisons were made on disk samples of leaf tissues, it is likely that those taken from leaves with systemic necrosis included groups of dying cells in which virus activity had decreased or disappeared, and also areas not invaded by the virus. The cells in samples from recovered leaves, on the other hand, seemed more uniformly invaded by the virus. These results suggest the following possibilities: a) that although the amount of active virus in necrotic areas was not significantly higher than in areas of recovered leaves as determined by the disk-sampling technique, the concentration in cells that had undergone necrosis might have reached a much higher level; and b) that although in some samples the amount of virus per cell is smaller in recovered leaf tissue than in cells that have shown necrosis, the total amount per area might be larger because of more uniform distribution.

The results described in this paper differ in part from those obtained by Fulton (5) and by Diachun and Valleau (4). These authors concluded that the concentration of the American tobacco streak virus was somewhat greater in recovered leaves than in leaves that showed systemic necrosis. Fulton, however, recognized that a small proportion of the total area of tissue was composed of dead cells in samples taken from necrotic leaves. Neither Fulton nor Diachun and Valleau indicated whether the comparisons were based only on samples of necrotic and recovered tissues from the same plant, or also between samples of recovered tissues from one plant and leaves showing early systemic necrosis from another plant.

Data obtained with Brazilian tobacco streak also suggest that recovery in this disease is associated with a mechanism that limits virus concentration in the cells below a certain level. That this mechanism begins to operate after the virus reaches the apical growing point there seems to be little doubt.

SUMMARY

In tobacco leaves with systemic necrosis caused by the Brazilian tobacco streak virus, the normal leaf tissue between the necrotic lesions is not completely invaded by the virus.

Tests of samples from leaves showing different

symptoms, taken at various intervals, indicated that there is a reduction in virus activity as the invaded leaves become old, especially in those that show necrosis.

Comparative tests indicated that the Brazilian tobacco streak virus reaches a concentration level higher in cells that show systemic necrosis than in cells of recovered leaves.

Recovery in Brazilian tobacco streak is also considered to result from a mechanism that limits virus concentration in the cells of newly formed leaves below a critical level, after the virus reaches the growing point of the plant.

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THE RATE OF PROGRESS AND DESTRUCTIVENESS OF CANKER STAIN OF PLANETREES[1]

James M. Walter,[2] Edgar G. Rex,[3] and Ray Schreiber

The literature on tree diseases presents very few definite statements concerning the destructiveness of otherwise well-known contagious pathogens. So far as the writers are aware, it affords no record of mortality rates for other tree diseases that could be classified with canker stain (5, 8) on the basis of means of transmission of the causal organism. It is hoped that the information herein on the destructiveness of canker stain will be of general interest to plant pathologists and at the same time serve to warn city foresters and tree wardens what to expect if they do not take the simple but necessary steps for control (3, 5, 6).

Canker stain (1, 4, 5, 6, 9) of planetrees is a highly infectious fungus disease of bark and wood, unique in the sense that the causal fungus is transmitted almost entirely by man. The pathogen, *Endoconidiophora fimbriata* (Ell. & Halst.) Davidson f. *platani*,[4] is probably indigenous to North America and occurs in very low incidence on planetrees in inaccessible areas seldom visited by man, but all available evidence indicates that it is of no economic importance where dependent upon natural agencies for transmission. Case-history records and observations covering the period 1939–1946 inclusive show that busy streets and good fences serve as highly effective barriers against the advance of the disease. The fungus is very readily transmitted by mechanical means, especially by pruning tools, and most efficiently in asphalt wound dressings (8). Observations on numerous isolated cases and small new outbreaks of canker stain made it clear, early in the intensive study of the disease, that a very high proportion of the primary infections started at pruning wounds made and treated according to standard techniques. A further characteristic of this disease is a relatively short incubation period: on the basis of external symptoms, infections may normally

[1] Accepted for publication December 21, 1951.

[2] Pathologist, Gulf Coast Experiment Station, Bradenton, Florida. Work reported here was performed while the senior author was employed as Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[3] Respectively, Supervisor and Associate Supervisor, Plant Pest Control, New Jersey Department of Agriculture, Trenton, N. J.

[4] The fungus involved here has been studied over a number of years by James M. Walter, Ross W. Davidson, W. J. Martin, and others, all of whom have found it morphologically indistinguishable from *Endoconidiophora fimbriata* and separable only on the basis of host reaction. It is accordingly established herewith as a new form:

Endoconidiophora fimbriata (Ell. & Halst.) Davidson f. **platani** Walter n. f.

Specie similis sed cortice et ligno Platani noxia.

Differing from the species only in its pathogenicity to *Platanus* spp.

Typified by specimen and culture isolated therefrom (Forest Pathology No. 1, 97462), collected by Richard Day and Horace Wester, Palisades Play Ground, Washington, D. C., August 14, 1951. Living culture deposited in the American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Holland. Type specimen and dried cultures in the Mycological Collections, Bureau of Plant Industry, Beltsville, Maryland.

The authors are indebted to John A. Stevenson and Edith Cash for their assistance in the technical description of the fungus.

be classified, by a worker familiar with the disease, as either positive or negative within 7 weeks during the leafy season; the uninitiated, however, might not recognize the typical infection until it had become 3 years old. The fungus kills the cambium and tender bark very quickly, and these tissues show a characteristic dark or "black" color; wood invaded by the fungus shows, at the advancing margins, characteristic radial color patterns in hues ranging from reddish-brown to bluish-black. In its advance into the wood, the pathogen is usually followed closely by saprophytes, especially wood-rot fungi (3, 6).

Canker stain is one tree disease that is being controlled by simple, practicable measures (3, 6, 7). However, the value of the well-cared-for trees that were infected before it was learned that the pathogen was being transmitted in pruning operations, probably exceeds $400,000, and the total loss due to the disease probably now exceeds $1,000,000. The principal host, London plane (*Platanus acerifolia* Willd.), has been accepted as one of the most valuable shade trees of central-eastern United States. Thousands of them have been planted in smoky, industrial cities since their unusual resistance to smoke, soot, and fumes was recognized. In the Southeast the native planetree or sycamore, *P. occidentalis* L., which is moderately susceptible to canker stain, is far more important as a shade tree, especially in small towns, than has been generally recognized.

MATERIALS AND METHODS.—A street-tree planting of London plane in the Highland Park district of Gloucester, New Jersey, is the source of the data herein. The trees were transplanted as commercial "standards" in 1915 by the real estate developer; and since the city has had no shade-tree commission, their care has been left to the residents except for line-clearance pruning by public utility companies. As previously reported (3), these companies of New Jersey and the line-clearance companies operating in the State began to practice the recommended precautions against transmission of the canker-stain fungus as soon as the information became available to them. Nothing was done to cause line-clearance crews to give the planting under consideration any special treatment. The first year that the use of antiseptic pruning technique could have influenced transmission of the fungus in the area concerned was 1942. A sharp reduction in the development of infections on branches and upper trunks was apparent by 1946, but this effect of disease control measures is believed to have occurred too late to significantly alter either rate of progress or net effect of the disease.

The canker-stain fungus probably was introduced to one or more trees of this planting by line-clearance pruning in 1926. In 1929 Dr. R. P. White of the New Jersey Agricultural Experiment Station observed two or three cases of the disease on Greenwood Avenue in Gloucester (3). It is now known, through inoculations and case-histories, that two to three years are required for an individual canker to develop to a noticeable stage. Thus it appears probable that the pathogen in this oldest known outbreak of canker stain had become established at least 20 and possibly 23 years before May 1949, i.e. in 1926.

All trees for two blocks on both sides of four successive avenues were carefully examined for evidence of canker stain in May 1940 and thereafter at intervals of 3 years.[5] Data for numbers of trees removed, dead, diseased, and healthy were recorded. All classifications were made during May, when external symptoms in a diseased tree are maximum for bark and wood invaded by the fungus during the preceding year, but before the appearance of any signs of new or current-season infections. All necessary probings were made with tools carefully disinfected with alcohol; and there was never any suggestion in later years that infections had occurred at wounds made during these examinations.

OBSERVATIONS.—The data, furnishing a record of the course of the disease in its oldest known center over 9 years, are presented in Table 1.

There are several possible ways of stating the indications of rate of progress and destructiveness of the disease afforded by the data. On the basis of the original number of trees in the planting, losses by May of the year indicated, and average annual increase in number of infected trees for the three years following, were: 1940, 43 and 3.1 per cent; 1943, 60 and 4.7 per cent; 1946, 74.8 and 1.7 per cent. Losses by May 1949 were 87.1 per cent. For 1940–1949 the increase in cases averaged 3.15 per cent, and the mortality rate was approximately 4.9 per cent. If we assume that the disease has been killing trees in the area for 20 years, the mortality rate has been 4.35 per cent each year. If we take 1926 as the beginning, however, and 23 years as the time during which 87.1 per cent of the trees were killed, the mortality rate has been 3.79 per cent per year.

Losses thus calculated include only trees removed and standing dead trees. No tree, no matter how badly diseased, was counted a loss until it had died, which in nearly all cases meant that cankers had girdled the trunk. There has been no known case of natural recovery from canker stain in the Philadelphia region; neither has there been, to our knowledge, a successful surgical removal of an early-stage infection from the trunk of a planetree, despite the practice of the most careful aseptic and antiseptic techniques. However, it appeared reasonable to wait until a tree had died to count it a loss, since we are concerned with the useful life-span of the host plant and know that a single infection usually requires 4 years to kill a tree of 1-ft. DBH and perhaps 10 years to kill one of 2-ft. DBH. The better-nourished trees of this

[5] The first examinations and records were made by the senior author after he had become sufficiently familiar with the disease through the necessary culturing, laboratory studies, and field inspections. Those in 1943 were made by Walter and Schreiber, those in 1946 by Walter and Rex, and those in 1949 by Rex and Schreiber.

TABLE 1.—*Numbers of London planes by classes with respect to canker stain in an outbreak in Gloucester, N. J., in May of 1940, 1943, 1946, and 1949*

Year and class	Street and row (West—East)								Totals	
	Greenwood		Rosalind		Sylvan		Orlando			
	W	E	W	E	W	E	W	E	Number	Per cent
Original population	37	38	34	32	36	34	34	34	279	
1940: Removed	26	24	12	12	5	7	10	12	108	38.7
Dead	0	2	3	1	1	2	1	2	12	4.3
Diseased	2	2	11	8	9	11	8	5	56	20.0
Healthy	9	10	8	11	21	14	15	15	103	37.0
1943: Removed	27	27	15	16	7	11	15	14	132	47.4
Dead	1	2	8	3	4[a]	8	6	3	35	12.6
Diseased	1	2	6	4	10	6	2	4	35	12.6
Healthy	8	7	5	9	15	9	11	13	77	27.4
1946: Removed	28	32	24	23	15	16	18	21	177	63.4
Dead	2	1	4	1	5	8	4	4	29	10.4
Diseased	1	3	5	2	7	6	6	5	35	12.6
Healthy	6	2	1	6	9	4	6	4	38	13.6
1949: Removed	31	34	31	25	23	28	25	30	227	81.4
Dead	0	1	2	1	4	2	4	2	16	5.7
Diseased	1	2	1	1	3	0	2	2	12	4.3
Healthy	5	1	0	5	6	4	3	0	24	8.6

[a] Includes one tree for which no evidence of the canker-stain fungus was found.

planting had attained 22-in. DBH by 1940. Multiple infections are common, however, especially on trees mutilated by boys in trying out their knives and hand-axes, and can kill a large tree very quickly. Several trees with multiple infections passed from the healthy to the dead category during a 3-year interval.

In 1940 the disease was more advanced on Greenwood Avenue than on the other avenues (Table 1). By 1949 the differential had been reduced considerably, and both Rosalind and Orlando Avenues had fewer healthy trees remaining.

Though the original number of trees in the planting has its advantages for calculation of rate of loss from the fixed population, it is not entirely satisfactory for consideration of the rate of advance of the pest, i.e., the development of new infections that change a tree from the healthy to the diseased category. Gradual reduction of the population in this way could greatly influence the transmission of the pathogen, through factors such as the increased distances between infected and healthy trees, and the reduced shade. Table 2 shows the increase in cases by 3-year periods, in percentages based on the numbers of trees unaffected by the disease at the beginning of each period. The data, both actual numbers of new cases and percentages, suggest that the disease accelerated most between 1943 and 1946, and that its rate of advance had slowed somewhat by 1949.

TABLE 2.—*New cases of canker stain in eight rows of planetrees, by 3-year intervals*

Avenue and row	Number of cases—percentage[a]		
	1940-1943	1943-1946	1946-1949
Greenwood W.	1 - 11.1	2 - 25.0	1 - 16.7
E.	3 - 30.0	5 - 71.4	1 - 50.0
Rosalind W.	3 - 37.5	4 - 80.0	1 - 100.0
E.	2 - 18.2	3 - 33.3	1 - 16.7
Sylvan W.	6 - 28.6	6 - 40.0	3 - 33.3
E.	5 - 35.7	5 - 55.6	0 - 00.0
Orlando W.	4 - 26.7	5 - 45.5	3 - 50.0
E.	2 - 13.3	9 - 69.2	4 - 100.0
Average percentage	25.14	52.50	45.84

[a] These percentages are based on the numbers of trees unaffected by the disease at the beginning of the three-year period, i.e., the numbers recorded as healthy at the end of the preceding interval. The actual numbers of unaffected trees are shown in Table 1 under the category healthy.

Comparison of the totals for living trees (diseased plus healthy) in Table 1 shows that the percentages of living trees found infected at the four inspection periods were as follows: 1940, 35.22; 1943, 31.25; 1946, 47.96; 1949, 33.33. As previously indicated, it was observed in 1949 that new infections ascribable to transmission of the fungus by line-clearance pruning were very few. On the other hand, there were, in 1946, an inordinate number of new infections at wounds made by boys in their play.

DISCUSSION.—Through shade-tree commissioners and other officials of the Philadelphia region it was learned that municipal budgetary allowances are made for an annual 3 per cent loss in shade trees; this embraces all causes for the removal of city-owned trees, including traffic accidents, which account for most casualties among curbside trees. Although losses due to canker stain were no doubt concerned to a considerable extent in this allowance, because the disease was killing planetrees in alarming numbers in some cities as early as 1933 (1), the writers do not believe that any such general deduction should be applied to the data in this paper for calculating the net loss due to canker stain. As shown in table 1, there was doubt about the disease being involved in the death of

only one of 243 trees. Moreover, the area selected is a relatively quiet, middle-class residential district, and none of the avenues is a main artery. The locality is believed to be truly typical of many communities in towns and cities of the central-eastern region in which canker stain may threaten to play havoc with the planetree population.

Fire and gas poisoning (2) were complicating factors in the death of a few trees during the 9 years of these observations, so that canker stain was not the sole cause. However, it appeared that these trees would have recovered had canker stain not been present. Thus the writers are convinced that canker stain is to be charged with nearly 100 per cent of the losses as recorded.

Trunk diameter is a factor in the rate at which the disease destroys the trees, since an affected tree does not ordinarily die until the cankers have completely girdled the trunk. The capacity of the fungus for tangential spread is low (4, 6, 9), and its annual advance in medullary rays seldom exceeds 3 in. Spread by actual growth of the fungus in the rays is the principal means of enlargement of old cankers and the development of true secondary cankers. This consideration of size of host stem on the rate of progress of the disease within the individual suscept indicates that, other factors remaining the same, the mortality rate due to the disease will be retarded a bit as the trees age and develop larger stems.

Because of the nature of the disease, the writers expect that a few trees of the planting recorded herein will be klendusic. The trend on Greenwood Avenue in recent years has suggested strongly that some trees have by chance failed to be inoculated and a few will very likely remain unaffected when the disease has subsided and "burned itself out" in the locality. A few klendusic specimens would have been expected in the planting even if line-clearance crews had not sharply curtailed transmission of the fungus, for the wires in such a development do not involve every tree.

By May 1943 the loss of trees (removed and dead) in the area studied amounted to 60 per cent, i.e., 167 of 279 trees (Table 1). In late 1943 Schreiber, as reported by Rex and Walter (3), estimated the total population of planetrees in Gloucester as 2,500 and the loss due to canker stain as 500 trees. In 1946 Walter (6) estimated the city's total loss from canker stain to be only 300 trees. This striking disparity in estimates made by two individuals familiar with the disease and the area is a good example of one kind of evidence that argues strongly for the collection of actual data on losses due to such diseases.

SUMMARY

In the oldest known center of the canker-stain disease of planetrees, infection is estimated to have started in 1926. Based on the original population, the mortality rate averaged 4.9 per cent and the increase in cases averaged 3.2 per cent per year for the period 1940–1949. By May 1949, 87.1 per cent of the trees had been lost to the disease and an additional 4.3 per cent were infected. The writers venture the opinion that a few of the trees in the planting will be klendusic.

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INHIBITION OF PLANT VIRUS INFECTION BY GROWTH PRODUCTS OF NEUROSPORA [1]

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It is apparent from recent work (4, 7) that a moderately large proportion of fungi produce growth products capable of inhibiting infection with certain plant viruses. The chemical nature of these products is unknown but their effect, in common with that of trypsin, plant saps, insect juices, normal sera, milk, and malachite green, not only occurs immediately on mixing with virus but also is reversible. Inhibition is therefore explicable either on the assumption of a reversible combination between inhibitor and virus or of alteration in susceptibility of host cells. Study of the inhibitor produced by *Trichothecium roseum* Link led to the conclusion that its activity is due to alteration in host susceptibility (5). The present study was undertaken to elucidate the mechanism of the inhibitory activity of the substances produced by *Neurospora sitophila* Shear & Dodge and *N. crassa* Shear & Dodge (4).

MATERIALS AND METHODS.—Five strains of *N. sitophila* obtained from B. O. Dodge (3), and differing in genetic factors for compatibility (Aa), production of protoperithecia (Pp), and production of conidia (Cc) were used in the first experiment. The wild type (APC) strain was used in subsequent experiments. *N. crassa* was obtained from the American Type Culture Collection. The fungi were grown in penicillin flasks on bacto-beef-cornmeal medium (4). After 21 to 28 days, contents of the flask were removed and passed through cheesecloth. The extract was centrifuged at 3000 r.p.m. for 15 min. and the supernatant filtered through a Seitz filter. The filtrate was stored at −5° F. in 100-cc. aliquots until used. Control solutions consisted of sterile bacto-beef-cornmeal medium which was centrifuged, filtered, and kept frozen in the same manner.

The viruses used were the type, Holmes' ribgrass (6), and tomato rosette (10) strains of tobacco mosaic virus (TMV) propagated in Turkish tobacco (*Nicotiana tabacum* L.), tobacco necrosis virus (TNV) propagated in necrotic type tobacco (a derivative of *N. digluta* Clausen & Goodspeed × *N. tabacum*). Southern bean mosaic virus (SBMV) propagated in the Bountiful variety of garden bean (*Phaseolus vulgaris* L.), and tobacco ringspot virus (TRSV) propagated in Turkish tobacco. Stock plants were harvested 21 days after inoculation, then ground in a meat grinder. The sap, which was pressed from the ground pulp, was clarified by centrifugation at 3000 r.p.m. for 15–20 min. Afterwards it was frozen in 30-cc. aliquots until used.

The inhibitory effect of the filtrates was determined by inoculating virus-filtrate mixtures onto leaves of appropriate test plants. All test plants were grown in composted soil in pots in a greenhouse. Inoculating was done by rubbing with a 3-in. square of cheesecloth, folded three time and moistened with the solution to be tested. Virus-filtrate mixtures were prepared by adding 4 cc. of the concentrated filtrate, or some known dilution of it, to 0.5 cc. of M/10 phosphate buffer at pH 7 and 0.5 cc. of an appropriate virus preparation. The control solution was prepared in the same manner except that sterile bacto-beef-cornmeal medium was substituted for filtrate.

EXPERIMENTAL WORK.—In the first experiment, 5 strains (APC, APc, ApC, aPc, and apC) of *N. sitophila* were used to determine whether or not genetic factors for compatibility, production of protoperithecia, or conidia inhibit or enhance synthesis of the inhibitory substance. Filtrates were mixed with 10^{-1} dilutions of TMV and SBMV prepared from plant sap in the manner described. The TMV solutions were tested on 8 half-leaves of *Nicotiana glutinosa* L. with the control solution on the opposite halves; the SBMV solutions were tested on 18 primary leaves of Kentucky Wonder wax bean plants with the control solutions on opposite leaves. The filtrates caused a reduction in infectivity somewhat higher than previously found (4)—85 to 99 per cent. The data provide no evidence of correlation between genetic composition of the fungus and production of inhibitor. Synthesis of the inhibitor is independent of the factors for compatibility, conidia and production of protoperithecia.

Thermostability of the inhibitor.—In the first of 2 experiments to determine the effect of heat upon the inhibitory agent of *N. sitophila*, 6 tubes containing 4 cc. each of filtrate and 6 tubes containing 4 cc. each of sterile medium were held in a water bath at 90° C. At various intervals 1 tube of medium and 1 of filtrate were removed and placed in a refrigerator. When all tubes had been removed and cooled, 0.5 cc. of a 10^{-1} dilution of SBMV and 0.5 cc. of M/10 buffer were added to each. Eighteen Kentucky Wonder bean plants were inoculated with the contents of each tube.

In the second experiment, 4 cc. of the filtrate and 4 cc. of sterile medium (the control) were autoclaved at 15 lb. pressure for 15 min. When the solutions had cooled, 0.5 cc. of TMV at a dilution of 10^{-2} and 0.5 cc. of M/10 buffer were added. The solutions were used to inoculate plants of *N. glutinosa* by the half-leaf method. There was no decrease in the amount

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of inhibition when the filtrate was heated for 1 hr. at 90° C. or when autoclaved at 123° C. for 15 min. The inhibitory agent is thermostable and therefore nonproteinaceous.

Essentially identical results were obtained when filtrate from cultures of *N. crassa* was used in similar experiments.

Dialyzability of inhibitor.—Filtrate from a culture of *N. crassa* and from noninoculated medium was placed in cellophane sausage casing, in 50-ml. volumes, and these were immersed in 1 liter of distilled water at 3° C. Other portions of the filtrates were mixed in an 8:1:1 ratio with buffer and virus and then dialyzed in like manner. The water was changed after 4 hr. and then at 8-hr. intervals. At various intervals, portions of both dialyzed and nondialyzed filtrates were mixed with buffer and virus in an 8:1:1 ratio and inoculated onto bean leaves. The dialyzed mixture was likewise tested on bean leaves. Dialysis of filtrate, whether mixed with virus before or after dialysis, had no effect on its ability to inhibit infection with SBMV.

Effect of pH on inhibitory activity.—Buffers prepared at various pH values by pairing stock solutions of 0.2M HCl, K phthalate, NaOH, KH_2PO_4, and H_3BO_3 in definite volume ratios and diluting to 200 cc. with distilled water were used to test the effect of pH on the inhibitory activity of filtrate of *N. crassa*. Mixtures containing 3.0 cc. of buffer, 1 cc. of sap expressed from plants infected with SBMV, and 6 cc. of filtrate (or of sterile bacto-beef-cornmeal medium) were inoculated onto 12 bean leaves. The pH of the mixtures was checked with a Beckman pH meter. In spite of increasing numbers of lesions with increase in pH value from 2.2 to 9.5, a characteristic property of SBMV (9), percentage reduction due to presence of the inhibitor was constant. Thus, the inhibitory agent of *N. crassa* is active over a wide pH range.

Test for a time reaction between virus and inhibitor.—To determine whether or not the inhibitory effect of the filtrates from *N. sitophila* and *N. crassa* would be increased by allowing them to remain in contact with virus for some time, mixtures containing SBMV and filtrate were allowed to stand at pH 7.0 and 3° C. For the test with *N. sitophila*, virus and filtrate were used at dilutions of 10^{-2}. For the test with *N. crassa*, the dilution was 10^{-1}. After various periods of standing, a sample was removed and used to inoculate leaves of Kentucky Wonder wax bean plants. A control solution containing virus at the same concentration at pH 7.0 was inoculated onto the opposite leaves.

Only 26 per cent inhibition of virus infectivity was obtained with *N. sitophila* filtrate at the beginning of the experiment, and degree of inhibition failed to increase with increasing length of contact between virus and inhibitor. Because a more concentrated solution of filtrate of *N. crassa* was used, inhibition was 76 per cent at the beginning of the experiment, but again degree of inhibition failed to increase significantly with increase in time of contact. The reaction between virus and inhibitor, if it occurs at all, must be completed in a very short time.

Effect of adding filtrate to plants at various intervals after inoculating with virus.—To determine whether or not the filtrate from *N. sitophila* would inhibit infection with SBMV when added to leaves previously inoculated with virus, SBMV at 10^{-2} dilution was rubbed over the primary leaves of 108 Kentucky Wonder bean plants and after various intervals (Table 1), the filtrate and sterile medium at 10^{-1} dilution were sprayed onto designated leaves of each of the plants. Leaves were immediately washed with water to prevent burning.

TABLE 1.—*Inhibitory effect of the filtrate applied at various time intervals after inoculation with SBMV*

Time after inoculation	Number of lesions on 18 bean leaves		Percentage reduction
	Control	Filtrate	
0 min.	1385	794	43
15 min.	1475	583	60
30 min.	1784	1418	21
60 min.	2618	2292	12
24 hr.	1992	1779	11
48 hr.	2098	1977	6

Inhibition was 43 per cent when filtrate was sprayed immediately after inoculation, but practically nil when spraying was delayed for 24 hr. (Table 1). Even with as little delay as 60 min. inhibition was negligible. Failure to obtain greater inhibition may have been due to washing immediately after spraying.

Similar results were obtained when filtrate from *N. crassa* was tested in like manner. When filtrate was rubbed over leaves immediately after inoculation, reduction in infectivity was 79 per cent; when rubbed 4 hr. after inoculation it was 55 per cent; when applied after 24 hr. no reduction was apparent.

Effect of adding filtrate to leaves at various intervals before inoculating with virus.—To determine the length of time leaves remain refractory to SBMV infection after treatment with the inhibitor of *N. sitophila*, 1 leaf on each of 126 Kentucky Wonder bean plants was rubbed with concentrated filtrate, the opposite leaf with sterile medium, and, after various in-

TABLE 2.—*Inhibitory effect of the filtrate applied at various time intervals before inoculation with SBMV*

Time before inoculation	Number of lesions on 18 bean leaves		Percentage reduction
	Control	Filtrate	
0 min.	670	122	82
10 min.	1875	187	90
30 min.	1145	304	75
1 hr.	439	250	43
4 hr.	975	482	50
24 hr.	1353	1223	9
48 hr.	2083	1655	20

TABLE 3.—*Inhibitory effect of filtrate applied to leaf surface opposite that used for inoculation with SBMV*

Time (in min.) after inoculation with SBMV	Number of lesions on 18 bean leaves		Percentage reduction
	Control	Filtrate	
0	819	565	31
15	855	456	45
30	1721	1053	39
45	1903	1280	33
60	1534	1228	20
75	1277	879	31

tervals, both leaves on sets of 18 plants were rubbed with a 10^{-1} dilution of SBMV (Table 2). Leaves remained refractory to infection for about 30 min. after treatment and then gradually regained susceptibility. At the end of 24 hr. they were about as susceptible as nontreated leaves.

Effect of applying inhibitor to under surface of leaves inoculated on upper surface with virus.—An experiment was made in such manner that filtrate did not come into contact with virus outside the plant, but only after both virus and filtrate had entered host cells. The upper surface of Kentucky Wonder bean leaves was inoculated with a 10^{-2} dilution of SBMV. After various intervals, filtrate at 10^{-1} dilution was sprayed onto the under surface in such a way that none of the filtrate came into contact with the upper surface.

The results (Table 3) show that spraying the under surface of inoculated leaves was about as effective in preventing infection by SBMV as spraying the upper surface (cf. Table 1). Inhibition was 30 per cent when leaves were sprayed immediately after inoculation or as long as 75 min. after inoculation. It is apparent that filtrate of *N. sitophila* is capable of inhibiting infection with SBMV even though it comes into contact with the virus only after both enter cells of the host.

Inhibitory effect with various host-virus combinations.—An experiment was carried out in which the inhibitory effect of Neurospora filtrate was determined for various host-virus combinations. Filtrate from both *N. sitophila* and *N. crassa* was used. Concentration of virus in the control and filtrate mixtures was 10^{-2}. Control and test solutions were inoculated onto 18 opposite leaves when bean plants were used and onto 8 half-leaves of the other host plants. Lesions were counted in from 5 to 7 days after inoculation.

It is apparent from the data (Table 4) that the inhibitory effect of filtrate on strains of tobacco mosaic virus is dependent to large extent upon the host plant used. In general, inhibition was high when plants of Early Golden Cluster bean and *N. glutinosa* were used, but there was little or none when plants of necrotic-type tobacco, *N. rustica* L., or *N. sylvestris* Spegaz & Comes were used. Inhibition of SBMV was essentially the same for the 2 varieties of bean plants. The filtrates caused no inhibition of infection of TRSV on either necrotic-type or Turkish tobacco leaves.

TABLE 4.—*Inhibitory effect of growth products of Neurospora on various host-virus combinations*

Virus and host	Number of lesions		Percentage reduction
	Control	Filtrate	
TMV			
Nicotiana glutinosa	775	22	97
Necrotic type tobacco	163	127	22
Early Golden Cluster bean	2895	32	99
N. rustica	178	67	37
TNV			
Kentucky Wonder wax bean	146	113	23
Early Golden Cluster bean	729	21	97
Necrotic type tobacco	1152	23	98
N. tabacum	155	137	11
TMV (tomato rosette)			
N. sylvestris	316	172	44
N. glutinosa	696	12	98
Early Golden Cluster bean	0	0	—
N. rustica	243	183	24
TMV (rib-grass)			
N. tabacum	205	173	15
N. sylvestris	250	155	38
N. glutinosa	523	37	93
TRSV			
Necrotic type tobacco	500	700	0
Turkish tobacco	490	650	0
SBMV			
Kentucky Wonder wax bean	3050	125	96
Early Golden Cluster bean	707	33	95

Results of the experiment suggest that the specific effect of the growth product is to alter host susceptibility rather than to decrease virulence of the virus. Stanley's (11) results on the inhibtory effect of trypsin on TMV were similar: he found that trypsin reduced the infectivity of TMV for *N. glutinosa* to a much greater extent than for Early Golden Cluster bean plants and concluded that trypsin altered susceptibility of the host plants. Bawden (1), on the other hand, suggested that some host-plant cells are better able to break the supposed combination between trypsin (or other inhibitory material) and TMV than others.

Separation by high-speed centrifugation.—An experiment was made to determine whether or not TMV could be separated by high-speed centrifugation from a mixture containing the inhibitory substance of *N. sitophila*, as was done in the case of the inhibitory substance of *T. roseum* (5). Two solutions, 1 containing a 10^{-1} dilution of TMV in M/10 phosphate buffer at pH 7 and the other containing a like concentration of virus and buffer and a 10^{-1} dilution of inhibitor, were centrifuged at 13,000 r.p.m. in a Serval SS-1a angle centrifuge for 2 hr. Six fractions, as follows, were tested for virus activity on *N. glutinosa* plants using 3 replications of a 6×6 Latin-square arrangement of test plant leaves.

TABLE 5.—*Relative infectivity of fractions obtained in the centrifugation experiment described in the text*

Fraction	Sum of logarithms of lesion counts on six leaves of *N. glutinosa*			
	Replicate No.			
	1	2	3	Total[a]
A	11.72	12.29	12.03	36.04
B	11.53	11.94	11.60	35.07
C	12.10	12.04	12.50	36.64
D	6.77	5.95	4.79	17.51
E	1.68	0.48	0.78	2.94
F	5.20	6.38	7.35	18.93

Analysis of variance:	Degrees of freedom	Sum of squares	Variance
Replicates	2	0.0	0.000
Treatments	5	52.06	10.412
Treatments × replicates	10	0.90	0.090
6 leaf positions	5	0.16	0.032
Leaf positions × replicates	10	1.81	0.181
Plants	17	2.41	0.141
Error	56	4.28	0.0738
Total	107	61.62	

[a] Differences greater than 4.63 between treatment totals are significant at the 0.01 level.

A. The pellet obtained by centrifuging the virus alone was resuspended and restored to original volume by addition of buffer.
B. The pellet obtained by centrifuging the mixture of virus and inhibitor was resuspended and restored to original volume by addition of buffer.
C. The original virus preparation without the inhibitor.
D. The original virus and inhibitor mixture.
E. The supernatant remaining after the pellet of fraction B had been removed.
F. The supernatant remaining after the pellet of fraction A had been removed.

The analysis of variance in Table 5 was calculated from logarithms of lesion counts on individual leaves. Since it was desired to test the significance of differences between various pairs of treatments, the standard error for difference was calculated: $\sqrt{0.0738} \times \sqrt{18} \times \sqrt{2} = 1.63$. The ratio of the difference between treatments to the standard error of the difference should exceed 2.84 to be significant at the 0.01 level.

The difference between fractions C and D shows that sufficient inhibtor was added to the mixture for the purpose of the experiment. (The inhibitor reduced the virus activity 91 per cent.) The supernatant from the solution containing virus alone (F) was found infectious, indicating that not all the virus was sedimented by centrifugation. However, A contained from 5 to 16 times as much virus as F, indicating that 83 per cent or more of the virus was sedimented.

Since A and B did not differ significantly, it can be concluded that 83 per cent or more of the virus present in the relatively noninfectious mixture was recovered by centrifugation. This conclusion is confirmed by the significant difference between B and D, which shows that virus sedimented from a mixture with inhibitor had more than 5 times the activity of the mixture. Presence of inhibitor in E no doubt accounts for the difference between E and F. The ease with which virus could be separated from a mixture with inhibitor by high-speed centrifugation suggests either that the virus failed to combine with the inhibitor or that the bonds in the presumed virus-inhibitor complex were extremely weak.

Effect of inhibitor on size of lesion.—It would seem fairly clear from the experiments so far reported that the inhibtory agent of *N. sitophila* exerts its effect by preventing infection. Presumably, infection could be prevented either by blocking entrance of virus into a susceptible region of the host plant or by tying up some constituent within cells required for virus multiplication. If the latter, it should be possible to inhibit virus multiplication in an infected leaf by continuous treatment with inhibitor. The following experiment was an attempt to do this.

A 10^{-3} dilution of TMV was inoculated onto 5 leaves of each of 33 plants of *N. glutinosa* which were then divided into 11 sets of 3 plants each. One set, as control, was not treated with inhibitor. The remaining 10 sets were sprayed from 1 to 10 times, respectively, with a 10^{-1} dilution of filtrate at intervals of 0, 1, 2, 3, 4, 5, 6, 72, 96, and 120 hr. after inoculation, the 0-hr. set receiving 1 spraying and the 120-hr. set, 10 sprayings.

Only the first spraying reduced the numbers of infections, the percentage reduction being constant (about 60 per cent) for the 10 treated sets. This is in agreement with the previous finding that the inhibitor is effective in preventing infection only if applied shortly after inoculation.

Size of lesion was not noticeably affected by application of filtrate even after 10 successive sprayings. Since increase in size of lesion is undoubtedly dependent upon multiplication of virus, it can be concluded that rate of multiplication within host cells was not altered by continued application of inhibitor. Therefore, it would seem that the inhibitory effect consists in preventing virus from becoming established within susceptible cells.

DISCUSSION.—Two general theories have been advanced to account for the mechanism by which certain materials inhibit infection by plant viruses: 1) that the inhibitor enters into a loose, but reversible, combination with virus (2, 8), and 2) that it alters susceptibility of host cells (11). Evdence obtained in the present investigation, comparable with that obtained for the inhibitory substance of *Trichothecium roseum* (5), supports the latter theory. It consists of the facts that 1) the presumed combination between the inhibitory substance of *N. sitophila* and virus, if it occurs at all, takes place instantaneously and can be broken by high-speed centrifugation; 2) that degree of inhibition varies with different kinds of host plants; and 3) that inhibition occurred when inhibitor and

virus were separately introduced into host plants with at least 7 layers of cells intervening between the points of entry of the 2 materials. It therefore seems reasonably clear that the inhibitor has little, if any, effect on the virus.

The desirability of determining the nature of the mechanism by which inhibitors alter susceptibility of host cells seems obvious. If they do so by combining with specific enzyme systems or metabolites required for virus reproduction, experiments can be devised to determine which systems are involved in reproduction. If, on the other hand, they do so merely by preventing entrance of virus into susceptible tissues, further study may still be of value in elucidating the nature of virus infection.

If the inhibitory substance of *N. sitophila* combines with cellular components required for virus multiplication, then treatment of infected plants should at least slow down the rate of multiplication. It was found that continued treatment of leaves of *N. glutinosa* with the inhibitory substance failed to reduce size of lesions produced by TMV, indicating that the virus multiplication rate was not appreciably altered. Therefore, it is unlikely that the mechanism of inhibition involves combination between inhibitor and specific cellular components. On the contrary, it is probable that activity of the inhibitor of *N. sitophila* is limited to interference with the infection process. The nature of this interference can be deduced by consideration of the effect of concentrated solutions of inhibitor on normal leaf cells. Concentrated solutions will cause visible burning of leaves to which they are applied, although solutions sufficiently concentrated to prevent infection may not necessarily be sufficiently concentrated to cause visible burning. Since impure solutions of the inhibitor were used, it is not definitely known that the component that causes burning is the one responsible for inhibition. However, there seems to be a correlation between ability of the filtrate to inhibit infection in specific host plants and ability to cause burning in the same plants. Concentrated solutions caused burning more frequently and more severe in leaves of *N. glutinosa* and bean than in leaves of Turkish or necrotic-type tobacco or of *N. sylvestris*. Inhibition of infection was high with the former but low with the latter (Table 4). Cells injured by the inoculation process are likely to be more sensitive to inhibitors than uninjured cells. As a result of these considerations, the hypothesis is advanced that trypsin, milk, insect juices, plant saps, fungal growth products, and similar materials inhibit infection by killing injured cells that otherwise could serve as entry points for virus.

SUMMARY

Filtrates of 5 different strains of *Neurospora sitophila* and 1 strain of *N. crassa* were tested for ability to inhibit infection by Southern bean mosaic, tobacco mosaic, and tobacco necrosis viruses. All filtrates were highly effective in reducing the number of infections, depending upon the host plant used. The inhibitory substance was most effective on plants of garden bean and *Nicotiana glutinosa*, least effective on plants of necrotic-type or Turkish tobacco.

The inhibitory agent was not inactivated by exposure to high temperature, suggesting that it is nonproteinaceous. It was effective immediately after mixing with virus, no time reaction being involved. It inhibited infection whether applied directly to the virus or to host-plant leaves either before or up to 30 min. after inoculation, and when applied to the leaf surface opposite that on which the virus was inoculated as well as to the same surface. Separation of virus from a noninfectious virus-inhibitor mixture was accomplished by high-speed centrifugation.

The evidence indicates that the inhibitory activity of the material from *Neurospora* is due to alteration in susceptibility of host cells. No evidence was obtained that multiplication of the virus could be stopped or even slowed down by application of inhibitor after infection had been established. The hypothesis is advanced that trypsin, milk, fungal growth products, and similar materials inhibit infection by killing injured cells that otherwise could serve as entry points for virus.

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STUDIES ON THE PATHOGENICITY OF SEVEN SPECIES OF PYTHIUM ON RED CLOVER SEEDLINGS [1]

J. E. Halpin, E. W. Hanson, and J. G. Dickson

While poor stands in red clover (*Trifolium pratense* L.) are usually most conspicuous the second year, substantial losses also occur the first year (5). Buchholtz (1) reported average seedling stands in field plots of only 56.2 per cent. Good management practices, such as use of good seed, careful preparation of seed bed, and shallow seeding, contribute greatly to the establishment of good stands, but even with the best agronomic practices, many seedlings die either prior to or soon after emergence.

Kilpatrick and Hanson (6) found that *Pythium* spp. were more commonly found on young plants than on older ones. Kreitlow et al. (7) isolated *Pythium* cultures from damped-off seedlings of several small seeded legumes including red clover and found that their isolates from red clover and alfalfa (*Medicago sativa* L.) were more pathogenic than the other isolates obtained. Buchholtz and Meredith (2) reported stand losses due to *Pythium* spp. greater in late seedings than in early ones. Buchholtz (1), in greenhouse tests using soil infested with *Pythium debaryanum* Hesse, found red clover more resistant to infection than alfalfa. He concluded that red clover's greater acid tolerance might be a factor. Jackobs (4) studied the factors affecting seed rotting caused by *Pythium* species in sweetclover with preliminary tests in red clover. Hoppe and Middleton (3) have reported that at least several species of *Pythium* occur in Wisconsin soils.

In general, information concerning pathogenicity of *Pythium* species on red clover is very limited. Pure culture work with known species of this fungus is almost completely lacking. Most of the earlier work has been done with *Pythium* infested soils or with unidentified isolates of the fungus. Consequently, critical descriptions of symptoms and information concerning differences between species have not been reported. The present study was made to obtain more specific information concerning the pathogenicity of various known species of *Pythium* on red clover seedlings.

MATERIALS AND METHODS.—Seven species of *Pythium* were used. These were: *P. debaryanum* Hesse, *P. irregulare* Buisman, *P. paroecandrum* Drechsler, *P. rostratum* Butler, *P. splendens* Braun, *P. ultimum* Trow, and *P. arrhenomanes* Dreschler. The first 6 species listed were obtained from Wisconsin soils. *P. arrhenomanes* is known to occur in Wisconsin soils but the isolate used in these investigations was obtained from a field in South Dakota. All the species were isolated from corn and all were identified by Doctor John T. Middleton.

Effect of temperature on radial growth was determined for each species over the range 0° to 36° C. Cultures were grown on potato-cerelose-agar (9) in Petri plates and the diameter of the colonies, less the diameter of the inoculum, determined 36 hr. after inoculation. Temperatures were kept constant by means of controlled temperature boxes. This study was repeated twice using 4 replicates for each trial.

In the preliminary pathogenicity studies, several methods were tried but the method that gave best results was that in which seedlings were grown in artificially infested sand in jelly glasses (Fig. 1). These glasses were 70 mm. in diameter at the top, tapering slightly towards the base, and 90 mm. in height. They proved to be excellent containers be-

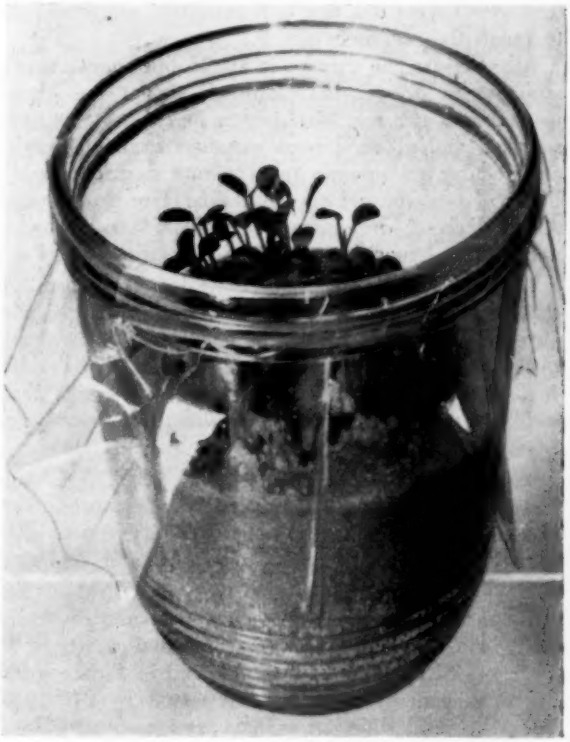

FIG. 1. Equipment used for testing the pathogenicity of *Pythium* species on red clover seedlings in the greenhouse.

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cause they provided adequate space for both root and top development of up to 100 seedlings without excessive crowding, required a minimum amount of greenhouse and storage space, were inexpensive and reusable, could be readily cleaned and sterilized, and permitted light to reach the seedlings.

The substratum consisted of white silica sand, agar, and a 1 normal Hoagland solution. The sand was sterilized and then mixed with potato-cerelose-agar (9) covered with thick mats of 5-day-old *Pythium* mycelia of the species to be tested. Agar and mycelium from one Petri plate culture was allowed for each jelly glass. Sand, agar, and fungus were thoroughly mixed in a large sterile container and equal quantities of the mixture placed in each glass, the amount being sufficient when tamped down to half fill a glass. The mixture was next moistened by adding the Hoagland nutrient solution down the inside edge of the glass with a pipette until the moisture present was sufficient to rise to the surface in the center of the sand by capillarity. Adequate checks were included in all experiments. These were prepared in the same manner except that the agar used in their preparation contained no fungus.

One hundred seeds were placed on the surface of the substratum, covered with a 1/16 in. layer of sterile sand, the surface remoistened with nutrient solution if not saturated by solution below, and the tops of the glasses covered with a 6-in. square sheet of cellophane. The cellophane prevented contamination and moisture loss from drying out, and still permitted sufficient light to reach the seedlings.

All seed used in this study originated from a single seed lot of Wisconsin Mildew Resistant red clover produced at Prosser, Washington in 1947. It was not scarified and all seed used was first examined under a binocular microscope to eliminate damaged seeds. Plating tests showed the lot to be free from fungi. Also, all seed was surface disinfected with alcohol and sodium hypochlorite immediately prior to use.

Duration of pathogenicity tests was 15 days. At the conclusion of each experiment, seedlings were carefully removed from the soil, washed, and the desired notes and measurements taken. The latter included stand counts, determination of length of shoot and root development of the seedlings, symptoms, and severity of injury.

Four temperatures, 16°, 20°, 24°, and 28°C. kept constant by means of controlled temperature greenhouses, were used in the pathogenicity trials. The experiment was repeated 3 times, using 4 replicates in each test.

RESULTS.—*Temperature studies.*—Results of studies on the effect of temperature on the radial growth of the 7 species of *Pythium* are summarized in Figure 2 and agree in general with those reported by Middleton (8) who used different isolates and also a different substratum. All species grew well over a wide range of temperatures and gave greatest diameter of growth at 28° C. While all species had the same optimum

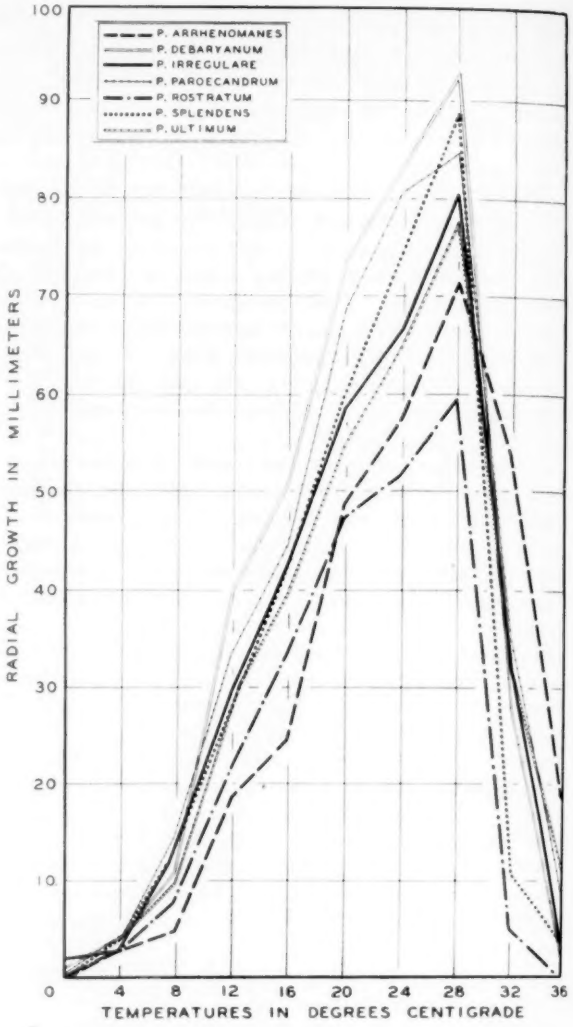

FIG. 2. Graphic representation of effect of temperature on the radial growth of 7 species of *Pythium* as determined on potato-cerelose-agar 36 hr. after inoculation.

and showed similar growth curves, there were distinct differences in response to temperature. *P. debaryanum* was the most rapid grower at temperatures ranging from 10° to 28° C., but mycelial development of *P. arrhenomanes* was greater at temperatures above 32° C. *P. rostratum* was slowest in mycelial development at temperatures of 20° to 36° C. while *P. arrhenomanes* was the slowest at 5° to 19° C.

Pathogenicity studies.—Three types of injury resulted from inoculation with Pythium: 1) pre-emergence killing, beginning as soon as the seed was sown and developing very rapidly so that seedlings were destroyed before they could emerge from the soil (Fig. 3A); 2) post-emergence damping-off, in which infection usually occurred prior to emergence but the rate of disease development was slower and seedlings emerged only to be killed soon thereafter (Fig. 3.B); 3) root rotting with varying degrees of stunting in

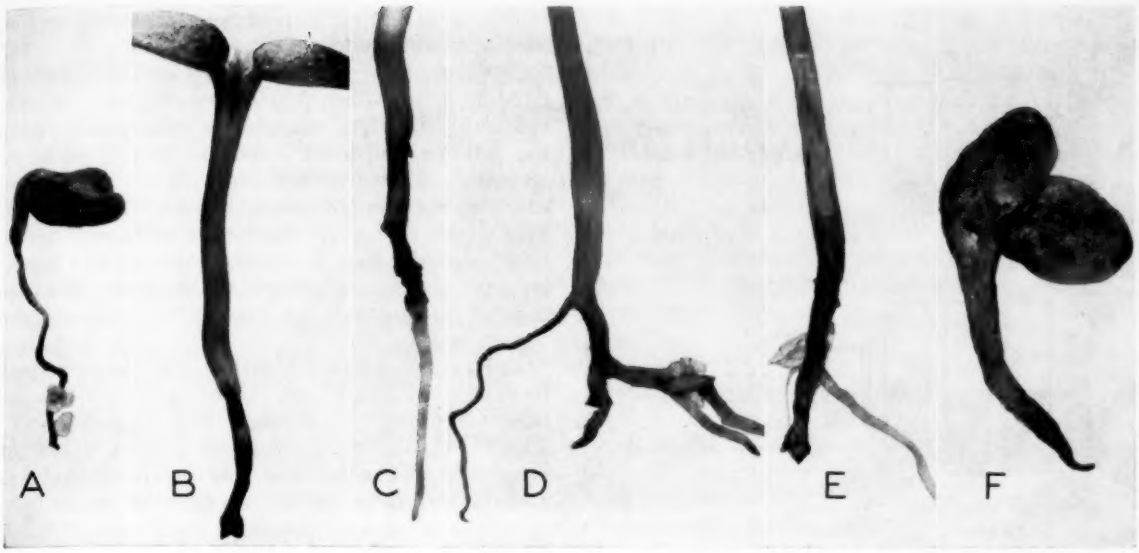

FIG. 3. Symptoms incited by *Pythium* spp. on red clover seedlings. A. Pre-emergence damping-off incited by *P. debaryanum*. B. Severe rotting of the primary root and lower part of the hypocotyl resulting in post-emergence damping-off, incited by *P debaryanum*. C. Complete destruction of the secondary roots and severe rotting of the upper part of the primary root and lower part of the hypocotyl, caused by *P. ultimum*. D. Rotting of primary and secondary roots, incited by *P. paroecandrum*. E. Severe rotting of the primary root and lower part of the hypocotyl, and a conspicuous spotting and streaking of the hypocotyl caused by *P. splendens*. F. Abundant conspicuous spots and streaks on cotyledons and hypocotyl caused by *P irregulare*.

which plants survived early seedling stages, some recovered and some died later (Fig. 3, C, D, and E).

The relative pathogenicity of the seven species studied is indicated in Table 1, and Figure 4. At all temperatures tested, *P. debaryanum* was the most virulent pathogen. It killed most plants prior to emergence, and the few seedlings that did emerge were very severely diseased.

P. rostratum was the only species completely nonpathogenic to red clover seedlings. It caused no discoloration of any kind to any part of the seedlings. Roots were white; seedlings were as healthy and advanced as the checks. Also, the fungus could not be isolated from plants grown in the presence of this species.

All species other than *P. rostratum*, were pathogenic on red clover. All produced definite discoloration of roots, hypocotyl, and cotyledons. Some caused seed rotting. Symptoms on each part of the diseased plants will be discussed separately.

On roots, the type of lesion and discoloration were similar but not identical for all species. Lesions ranged from tan to chocolate brown in color, were necrotic and somewhat sunken, with damage to the cortex evident. All pathogenic species showed ability to destroy the root tip. Severity ranged from slight discoloration of localized areas to complete destruction of the entire root system, when seedlings wilted and died. Although all 6 pathogenic species were able to destroy completely the roots of infected plants, the frequency with which each species did this was about proportional to its ability to reduce stand (Table 1).

TABLE 1. —*Effects of 7 species of Pythium on stand and disease development of red clover seedlings grown in the greenhouse at 4 different temperatures for 15 days*

Species of Pythium	Average stand in per cent and disease severity rating at indicated temperatures				
	16°C.	20°C.	24°C.	28°C.	Average for four temperatures
None (check)	74 (0)[a]	77 (0)	76 (0)	76 (0)	76 (0)
P. rostratum	73 (0)	74 (0)	76 (0)	74 (0)	74 (0)
P. arrhenomanes	70 (1−)	73 (1)	66 (1)	67 (1)	69 (1)
P. paroecandrum	65 (1+)	59 (1+)	49 (1+)	59 (2−)	58 (1+)
P. irregulare	49 (3)	35 (3)	37 (3+)	33 (3+)	38 (3+)
P. ultimum	33 (2)	40 (2+)	35 (2)	36 (2)	36 (2)
P. splendens	28 (2+)	26 (2+)	19 (3−)	15 (2+)	22 (2+)
P. debaryanum	3 (4−)	4 (4−)	4 (4−)	6 (4−)	4 (4−)

[a] Severity ratings are given in parenthesis and based on emerged seedlings only; 0 = no disease and 4 = all plants killed.

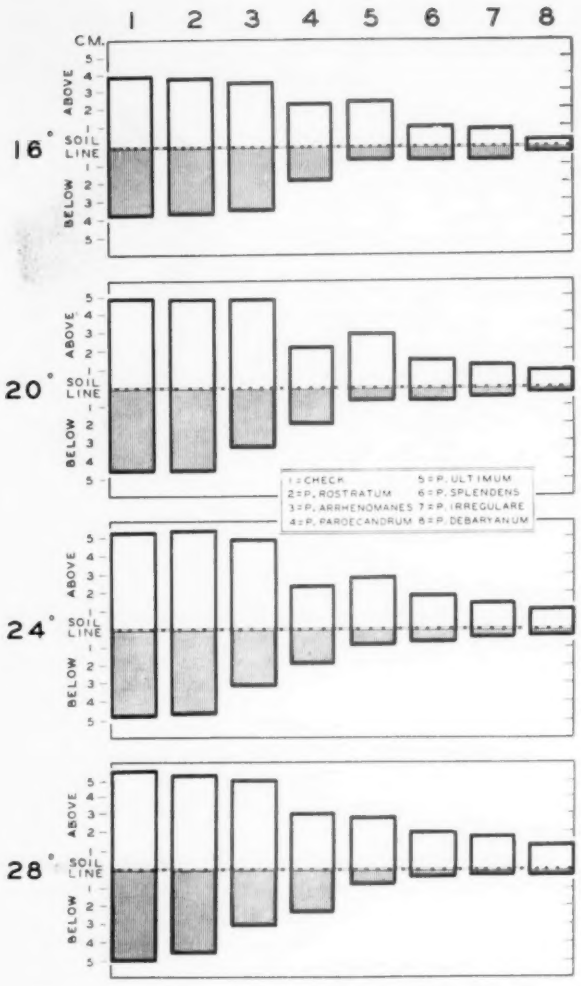

FIG. 4. Length in centimeters of above and below ground parts of red clover seedlings still alive 15 days after inoculation with 7 species of *Pythium* at 4 different temperatures.

Cotyledons and hypocotyls of seedlings infected with *Pythium* spp. commonly showed characteristic spotting (Fig. 3,F). On the cotyledons spots were slightly sunken, circular or irregular, necrotic areas, pinpoint up to 2 mm. in diameter, and very conspicuous. On the hypocotyls, they were similar but usually included many linear dark streaks and showed evidence of causing cortical decay. Spotting was due to infection prior to emergence and did not extend to the epicotyl which develops subsequent to emergence. *P. irregulare* produced the most intense spotting, while *P. arrhenomanes* and *P. paroecandrum* produced the least.

One phase of pre-emergence killing was seed rotting. This was characteristic for *P. debaryanum*, *P. irregulare*, *P. splendens*, and *P. ultimum*, but not for other species studied. Rotted seeds were wet, soft, and tannish in color. This type of rot probably began at the time the seeds absorbed water prior to germination. Hard seeds did not rot. Pre-emergence killing was similar in type for all species. It was due to a rapid infection of all parts of the germinating seedling causing a soft, dark rot.

Pythium paroecandrum and *P. arrhenomanes* differed from the other 4 pathogenic species in being less virulent. Both caused less reduction in stand, less stunting, and fewer lesions on the cotyledons and hypocotyls. They produced a tannish root discoloration with the more severe rotting limited to the root tips. This was in contrast to the 4 more pathogenic species which caused severe chocolate brown lesions extending over the greater portion of the roots. Moreover, these 2 species did not prevent the production of branch roots.

The 3 types of injury described were closely related; they involved mostly degrees of severity. If conditions for disease development were favorable and a virulent species of *Pythium* was present, much pre-emergence killing resulted. If the conditions were slightly less favorable or a somewhat less virulent species was present, the disease progressed less rapidly and the seedlings emerged before being killed. In other cases, the injury was still less severe and the seedlings were not killed but frequently were stunted (Fig. 4). Extent of injury ranged from none to complete killing of the seedlings prior to emergence. Severity ratings were recorded for each species at each temperature (Table 1). These ratings were used to estimate relative amounts of injury to surviving plants. Cotyledon and hypocotyl lesions were not rated as important as damping off, stunting, and severity of root lesions.

DISCUSSION.—Results reported in this paper were obtained under pure culture conditions and consequently may or may not indicate the relative pathogenicity of these fungi under field conditions. However, all the species studied occur in Wisconsin soils, where several of them, especially *Pythium debaryanum*, have been frequently isolated from diseased red clover seedlings grown in the field, and it is likely that some play an important role in stand reductions occurring the first few weeks after seeding. Hoppe and Middleton (3) have reported an association between soil type and the presence of certain species of *Pythium*. They suggested also that the association is probably influenced by crop sequence. More work on these phases of the problem would seem profitable.

It is possible that seed treatment with fungicides may protect seeds and young seedlings against infection. This phase of the problem likewise needs further investigation.

There is a possibility that the symptoms observed on the cotyledons and hypocotyls of red clover seedlings infected with *Pythium* will prove to be a useful character for preliminary diagnosis in the field. The very conspicuous spotting described is not usually produced by most other fungi that have been checked, and while not every plant infected with *Pythium* will show this symptom, many of them do. The percentage of plants showing the symptom appears to be correlated with the pathogenicity of the species. The

less virulent species of *Pythium* produce the least spotting.

SUMMARY

Pythium debaryanum, P. irregulare, P. splendens, P. ultimum, P. paroecandrum, P. arrhenomanes, and *P. rostratum* were compared for relative pathogenicity on red clover seedlings. *P. debaryanum* was most pathogenic followed by *P. irregulare, P. splendens, P. ultimum, P. paroecandrum* and *P. arrhenomanes* in descending order. *P. rostratum* was non-pathogenic.

The optimum temperature for radial growth of mycelium on potato-cerelose-agar plates for all species was 28°C. All pathogenic species incited disease over the entire range of 16° to 28°C., being usually somewhat more pathogenic at the higher temperatures.

Pre-emergence killing, post-emergence damping-off, root rotting, spotting of the cotyledons and hypocotyls, as well as stunting were common symptoms produced by all pathogenic species. The characteristic spotting of the cotyledons and hypocotyls might be useful in preliminary field diagnosis.

A jelly glass technique for studying the relative pathogenicity of fungi attacking clover seedlings proved very satisfactory for this purpose.

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NOCARDIA VACCINII N. SP. CAUSING GALLS ON BLUEBERRY PLANTS [1]

J. B. Demaree [2] and N. R. Smith [3]

In a previous report (5) on the occurrence of bud-proliferating galls on blueberry (Vaccinium spp) the causative organism was tentatively identified as an actinomycete, as characterized in the fifth edition of Bergey's Manual (1). Later studies on the morphology and cultural characteristics of the organism showed that it belongs to the genus *Nocardia* in the family *Actinomycetaceae* (2).

This unusual blueberry disease was first observed in 1944 on plants grown as part of the blueberry improvement program at the U. S. Plant Industry Station, Beltsville, Maryland. The plants were seedlings resulting from crossing two blueberry species, *Vaccinium australe* Small and *V. ashei* Reade. Among several lots of seedlings, totalling over 1,700 individual plants, the incidence of obvious natural infection ranged from 8 to 70 per cent, averaging 26 per cent.

For the first 6 months the seedling plants grew in pots filled with a soil composed largely of unsterilized leaf mold obtained from a wooded area where the black huckleberry, *Gaylussacia baccata* (Wang.) K. Koch, grew profusely. This species was affected with witches'-broom of undetermined cause. Although *Nocardia* was not isolated from the witches'-broom, it seemed probable that the latter was the source of the inoculum that caused the blueberry galls. This hypothesis was strengthened by the fact that when a different potting material was used no galls appeared on the blueberry seedlings.

DESCRIPTION OF THE GALLS.—Galls occurred naturally on plants at or immediately below the ground line. None was observed on stems above ground or on branches, and rarely on roots. Galls varied from ½ to 2 ins. in diameter. Bud proliferations were of common occurrence and usually abundant (Fig. 1). The abnormal buds generally aborted during an early stage; others grew into thin, weak shoots 1 to 6 ins. high, forming a witches'-broom effect at the base of the plants and finally dying.

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FIG. 1. Bud-proliferating gall caused by *Nocardia vaccinii* n. sp. on naturally infected blueberry plant.

Most galls produced abnormal spindly shoots either over the entire surface or on the upper portion. Young gall tissues were white and soft. As the galls aged they became brown to black and hard. Production of bud proliferation is characteristic of this type of gall and readily distinguishes it from the so-called "phomopsis gall" described by Brown (3), now considered to be caused by a strain of *Agrobacterium tumefaciens* (6).

ISOLATIONS AND INOCULATIONS.—During the early stages of this investigation, about 400 unsuccessful attempts were made to isolate *Agrobacterium tumefaciens* from the galls and the short thin shoots growing from them. Various fungi and bacteria grew from the tissue plantings on nutrient media; some of these were used to inoculate injured plants known to be susceptible to infection by the crown gall organism. The wounds healed normally and no galls formed.

Later several field-grown plants bearing galls were transferred to a warm greenhouse. The plants grew vigorously and some of the galls enlarged. One gall which made an extensive new excrescence was selected for further isolation trials. Many tissue plantings were made from the inner portions of the gall and of the adventitious shoots. Of several organisms which grew from the tissue planting, twenty-three different bacterial, or bacterial-like, cultures were saved for further studies. Each of these isolates and also a stock culture of *Agrobacterium tumefaciens* were inoculated into each of five blueberry plants. Two of the isolates (BG 7 and BG 19) caused galls typical of those earlier observed on field-grown plants. Isolate BG 7 was lost, but BG 19 remained alive and was used to inoculate numerous plants. The results of the inoculations are in Table 1.

TABLE 1.—*Results of inoculating blueberry plants with Nocardia (BG 19)*

Date inoculated	Number plants inoculated	Number plants infected [1]
May 1945	10	7
April 1946	16	14
May 1946	46	30
June 1946	20	17
August 1946	20	13
April 1948	10	9
July 1950	5	3
August 1951	12	9
Total	139	102

[1] Per cent infected, 73.3.

After the trial inoculations with twenty-four cultures, all plants used were grown in autoclaved soil. Usually they were young seedlings of *Vaccinium australe*, *V. ashei*, or their hybrids. The inoculum was placed against injured stems immediately below the soil surface. To show whether injury was necessary, one lot of twenty uninjured plants was exposed to the organism by applying it carefully to the stem. Only one plant formed a gall and that may have inadvertently been injured. Inoculations above ground on stems, branches, and buds were unsuccessful.

One lot of fourteen plants was grown in unsterilized leaf mold obtained from the wooded area mentioned earlier and was injured immediately below the soil level, but no inoculum was applied. Three of the fourteen plants formed galls, thus confirming the hypothesis that leaf mold may have been involved in the infection of the field-grown plants.

The organism was reisolated twice from galls formed as the result of inoculating plants with isolate BG 19. Transfers from one reisolate infected seventeen of twenty blueberry plants inoculated and the other infected nineteen of twenty plants used.

A few species besides blueberry, including aster, azalea, impatiens, kalanchoe, olive, strawberry, and vinca were inoculated with isolate BG 19. Usually only five plants of each species were used, but when twenty-five azalea plants were inoculated, none showed evidence of infection.

A preliminary study of BG 19 indicated that it might be identical with Jensen's *Proactinomyces minimus* (7), now known as *Nocardia minima* (2). A culture of this was obtained from the American Type Culture

Collection (No. 8674) and twenty-five young blueberry plants were inoculated. As a control, twelve blueberry plants were inoculated with BG 19. After 6 weeks, nine of the twelve plants inoculated with BG 19 had galls (Table 1), but none of the twenty-five inoculated with Jensen's organism showed abnormal growths.

CHARACTERISTICS OF THE CAUSATIVE ORGANISM.—The original isolate BG 19 and the two strains (V1b and V2a) isolated from a gall caused by BG 19 were studied in detail and found to be the same organism. Their characterizations follow:

Vegetative forms: Rods and filaments 0.4μ to 0.8μ in diameter, granular appearance when stained and eventually breaking up into bacillary forms (Fig. 2), few cells acid-fast. Fat was demonstrated by staining with Sudan black B.

Nutrient agar: Growth poor, slow, granular, grey, sometimes pinkish in old cultures.

Glucose-nutrient agar: Same as nutrient agar.

Czapek's agar: Growth scant, grey.

Potato-yeast-mannitol agar: Growth abundant, fluffy, grey to orange.

Soybean agar: Growth, if any, restricted.

Potato: Growth slow, spreading, raised, grey.

Milk agar plate: Growth dry, raised, grey with orange spots; no hydrolysis of the casein.

Gelatin-nutrient agar plate: Dry, ribbon growth; no hydrolysis of the gelatin.

Starch-nutrient agar plate: Dry, ribbon, pinkish to orange growth; starch hydrolyzed.

Fermentation reactions: On a basal agar with ammonia as the source of nitrogen, acid was formed from glucose, sucrose, glycerol, and mannitol; reactions variable with arabinose and xylose; no growth on lactose or sorbitol.

Nitrates: Slowly reduced to nitrites.

Citrates: Utilized to a limited extent.

Paraffin: Utilized.

Indole: Not utilized.

Temperature relations: Growth best at 25° to 28° C.; inhibited at 32°; none or very scant at 37°.

Antibiotic activity: None against *Escherichia coli*, *Micrococcus* spp., *Bacillus cereus*, and *B. subtilis*.

Distinctive characters: Resembles *Nocardia minima* (Jensen) Waksman and Henrici (2) but differs from it in the following respects: Ferments glycerol and mannitol and sometimes arabinose and xylose; reduces nitrates to nitrites; utilizes citrates; and causes formation of bud-proliferating galls on blueberry plants.

DISCUSSION.—Taxonomists are always confronted by the question as to what constitutes a species. Some genera of bacteria have been studied very carefully with the result that certain entities are recognized as valid species. Other genera, or groups, are not as clearly characterized. The International Code (4, p. 289) recognizes this situation: "The definition of each of these categories (ranks) varies, up to a certain point, according to individual opinion and the state of the science."

The differences noted above between the blueberry *Nocardia* and *N. minima* are considered by the writers to be of sufficient importance to warrant the designation of the former as a new species. It is true that it would be advisable to study more isolations of BG 19 and of *Nocardia minima* to establish the limits of the variations that occur in these species. On the other hand, the differentiating characters, especially pathogenicity, would seem to warrant the separation of the two and the designation of BG 19 as a new species. The name suggested is *Nocardia vaccinii* n. sp. A culture was deposited in the American Type Culture Collection, 2029 M Street, N. W., Washington 6, D. C., under No. 11092. Specimens of artificially-infected galls caused by isolate BG 19 were deposited in the mycological collections of the U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland, under No. 71592.

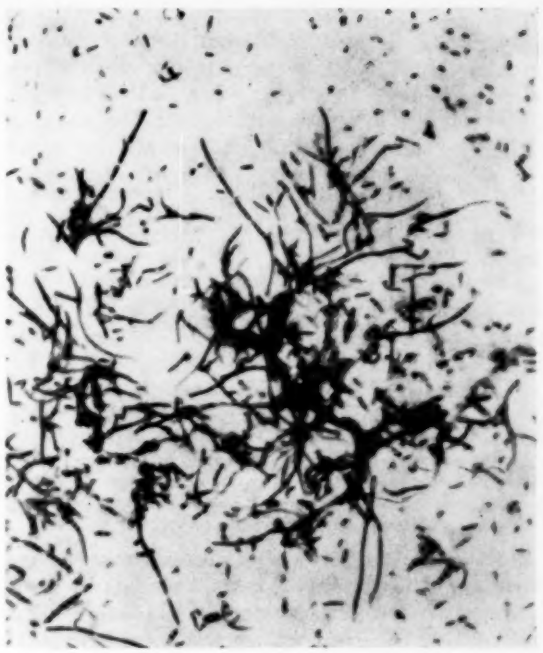

FIG. 2. *Nocardia vaccinii* n. sp. from potato-yeast-mannitol agar, showing filaments breaking up into bacillary forms. Approximately ×1000.

As far as the writers know, this is the first time that a species of the family *Actinomycetaceae* has been found to cause a plant tumor.

SUMMARY

Bud-proliferating galls that formed a witches'-broom effect just below the ground line occurred on about one-fourth of the young blueberry plants initially grown in plots containing a special soil composed partly of leaf mold obtained from a wooded area near Beltsville, Maryland. A species of *Nocardia* isolated from such a growth produced typical galls on blueberry plants. From artificially infected plants, two reisolates that also produced typical bud-proliferating galls were obtained.

The organism resembles *Nocardia minima* more than any other described species, but it can be separated from the latter by the utilization of glycerol, mannitol, and citrates; the reduction of nitrates; and the formation of bud-proliferating galls on blueberry plants.

The name proposed for this organism is *Nocardia vaccinii* n. sp.

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COMPARATIVE TRANSMISSION OF BEET-MOSAIC VIRUS BY FOUR APHID SPECIES [1]

Edward S. Sylvester [2]

Beet-mosaic virus, as well as several other nonpersistent aphid-borne viruses, is transmissible by several vector species, with varying degrees of efficiency (2,5). Transmission efficiency ratings were obtained by two methods, first by using relatively long infection and test feeding periods without a controlled pre-infection feeding starvation period (2), and second by fasting the insects prior to a short infection feeding (5).

Experiments reported herein were undertaken to increase knowledge regarding the mechanism of transmission of beet-mosaic virus and the causes for varying vector efficiency. Ability of various aphid species to pick up and inoculate virus during short feeding periods such as probably obtain while winged aphids search for favorable hosts in the field, was tested. Effects of pre- and post-infection feeding starvation periods such as exist during aphid population dispersal movement were studied. Similar studies on 1 species, the green peach aphid, *Myzus persicae* (Sulzer) have been previously reported (3). The species served here as a standard for comparison.

MATERIALS AND METHODS.—Four aphid species were used, *viz.*, the green peach aphid, the foxglove aphid, *M. solani* (Kalt.), the crescent marked lily aphid, *M. circumflexus* (Buckt.), and the celery aphid, *Aphis apii* Theob. (= *A. helianthi* Monell ?). Noninfective colonies of the green peach aphid were maintained on pak choi. Noninfectives of the other 3 species were colonized on celery. No difficulty in feeding the aphids on sugar beets was encountered during the short periods of time used. Insects were transferred with a camel's hair brush. Infection and test feedings were timed with a stop-watch. Starvation was either in vials (groups of insects) or in gelatin capsules (individual aphids).

Test plants were transplanted seedling beets inoculated while in the cotyledon or early leaf stage. Plants were fumigated with nicotine at the end of the test feeding before being put into the greenhouse for incubation. The results are those obtained in 30 replications, 1 plant per treatment, in all experiments. The sequence of species used in the acquisition and inoculation threshold trials was randomized.

RESULTS. *Acquisition threshold.*—To determine the acquisition threshold the following infection feeding intervals were used: 5, 10, 15, 20, 25, and 30 sec. Individuals of the 4 aphid species were starved before infection feeding. The ranges (means) for the pre-infection feeding starvation period for the 4 species were: green peach aphid, 0.5-4.8 (1.4) hr.; foxglove aphid, 0.5-3.9 (1.2) hr.; lily aphid, 0.5-3.1 (1.3) hr.; celery aphid, 0.5-3.4 (1.4) hr. Following infection feeding the individuals were placed on healthy seedlings. The ranges (means) for test feeding periods of the 4 species were: green peach aphid, 0.6-1.9 (1.2) hr.; foxglove aphid, 0.9-2.3 (1.4) hr.; lily aphid, 0.6-2.5 (1.4) hr.; celery aphid, 0.7-2.1 (1.2) hr.

TABLE 1.—*Results [a] of trials to determine the acquisition threshold of beet-mosaic virus, with starved single apterae of 4 aphid species, a controlled variable infection feeding, and a mean test feeding of 1.3 hours.*

Species	Infection feeding period in seconds						
	5	10	15	20	25	30	Total
Myzus persicae	0	3	15	20	15	13	66
M. solani	0	2	9	9	12	6	38
M. circumflexus	0	0	2	0	4	1	7
Aphis apii	0	0	2	2	4	3	12
Total	0	6	28	31	35	23	123

[a] Numbers in the columns indicate the number of plants infected of 30 inoculated.

None of the species picked up virus from an infected plant in 5 sec. (Table 1). Two species, the green peach aphid and the foxglove aphid, acquired virus in the 6-10-sec. feeding. All species obtained an infective dose during the 15, 25, and 30-sec. intervals. The best

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performance of the green peach aphid was at the 20 sec. interval, while the other species were most efficient at the 25 sec. interval. A slight decline in efficiency of all species occurred during the 30-sec. interval. While there was a general rise in efficiency of acquisition from the shortest effective feeding interval to an optimum for each species, the level of infectivity varied greatly between species. Apparently the 4 species acquired virus in a similar manner, but at slightly different rates, and differed greatly in their overall ability to obtain virus during short infection feedings. The most efficient species was the green peach aphid. The foxglove aphid was intermediate, while the lily and the celery aphids were the least efficient, and little difference existed between the latter 2.

Inoculation threshold. The method of determining the inoculation threshold was similar to that used for the acquisition threshold. Test feeding periods were 5, 10, 15, 20, 25, and 30 sec. A mean infection feeding period of approximately 20 sec. was used for the green peach aphid, while 25 sec. was used for the other three species. The ranges (means) of infection feeding times for the 4 species were: green peach aphid, 15-25 (19.5) sec.; foxglove aphid 20-25 (24.8) sec.; lily aphid, 20-25 (24.9) sec.; celery aphid 22-30 (25.0) sec. The ranges (means) for the pre-infection feeding starvation period were: green peach aphid, 0.5-4.4 (1.4) hr.; foxglove aphid, 0.4-3.1 (1.6) hr.; lily aphid, 0.5-3.3 (1.7) hr.; celery aphid, 0.6-3.5 (1.7) hr.

Both the green peach and the foxglove aphids inoculated plants during a 10 sec. interval, the other two species during a 15 sec. interval (Table 2). Maximum infection was obtained with the green peach and foxglove aphids during the maximum test feedings (25-30 sec.). Number of infections obtained with the lily and celery aphids was too low to permit generalization as the test feeding intervals used were evidently of insufficient length to measure properly actual infectivity of the species. Improvement in performance can be gained by increasing either the number or length of the test feeding (3).

TABLE 2.—*Results* [a] *of trials to determine the inoculation threshold of beet-mosaic virus, using starved single aphid apterae of 4 aphid species, a 15 to 30 second infection feeding, and a variable test feeding period.*

Species	Test feeding period in seconds						
	5	10	15	20	25	30	Total
Myzus persicae	0[b]	4[b]	2[b]	3	5	5	19
M. solani	0	1	1	1	5	4	12
M. circumflexus	0	0	1	1	1	0	3
Aphis apii	0	0	1	1	0	2	4
Total	0	5	5	6	11	11	38

[a] Numbers in columns are number of plants infected of 30 inoculated.

[b] One plant in series died, so number is that infected out of 29.

Pre-infection feeding starvation. Numerous studies on aphid transmission of nonpersistent viruses have shown that vectors are better at acquiring virus if starved before a short infection feeding (1, 3, 4, 5, 6). Reports (1, 4, 5), have also indicated an increase in benefit, up to an optimum, as the starvation period is lengthened. In determining influence of the preliminary starvation period, individuals of the 4 aphid species were starved for 0, 5, 10, 15, 30, 60, and 240 minutes prior to being given a short infection feeding, after which they were transferred to test plants. The ranges (means) for the infection feedings were: green peach aphid, 12-25 (17.3) sec.; foxglove aphid, 10-25 (22.6) sec.; lily aphid, 12-25 (23.1) sec.; celery aphid, 15-30 (24.1) sec. The ranges (means) for the test feedings were: green peach aphid 0.8-5.3 (1.8) hr.; foxglove aphid, 0.9-4.7 (1.6) hr.; lily aphid, 0.9-4.4 (1.6) hr.; celery aphid, 1.0-5.8 (1.8) hr.

TABLE 3.—*Results* [a] *of trials to determine influence of length of pre-infection feeding starvation on vector efficiency. Single aphid apterae of 4 species were used. Infection feeding periods varied from 10 to 30 sec., and the test feeding period had a mean value of approximately 1.6 hr.*

Species	Pre-infection feeding starvation time in minutes							
	0	5	10	15	30	60	240	Total
Myzus persicae	1	3	6[b]	3	7[b]	12	9	41
M. solani	1	3	4	1	6	9	6	30
M. circumflexus	0	0	0	2	0	2	0	4
Aphis apii	1	1	1	0	2	0	1	6
Total	3	7	11	6	15	23	16	81

[a] Numbers in columns are number of plants infected out of 30 inoculated.

[b] One plant in series died, so number is that infected out of 29.

Results (Table 3) obtained with the green peach and foxglove aphids indicated that a starvation period of 5 min. was enough to increase efficiency and that longer periods up to and including an hour were of additional benefit. Apparently little gain in efficiency resulted from prolonging the period beyond a 60 min. interval. The data do not indicate a similar increase in efficiency of transmission by the lily and celery aphids.

Previously published results (3) indicated that increase in efficiency of transmission of beet-mosaic virus by the green peach aphid was not accomplished by increasing the preliminary starvation period beyond 5 min. Results in the present paper suggest that starvation periods up to an hour are increasingly effective. No reason for the discrepancy is apparent, unless the high level of transmission in the earlier work mitigated the expression of small differentials resulting from varying the length of the preliminary starvation period.

Post-infection feeding starvation. Various published results have shown that transmission of some nonpersistent viruses is reduced by subjecting vectors to a period of starvation following a short infection feeding (1, 3, 4, 5) and that virus loss also occurs during feeding (1, 5, 7). To date, data are too few to warrant definite comparison of rates of loss during starvation with those during feeding.

To test effects of post-infection feeding starvation on efficiency of transmission, individuals of the 4 aphid species were starved, allowed a short infection feeding, and again starved for intervals of 0, 5, 10, 15, 30, 60 and 240 min. before being placed on a healthy plant. During the experiment, the ranges (means) for infection feeding were: green peach aphid, 13-21 (18.3) sec.; foxglove aphid, 15-25 (22.1) sec.; lily aphid, 15-26 (23.4) sec.; celery aphid, 10-29 (23.9) sec. The values for the preliminary starvation were: green peach aphid, 0.5-5.2 (1.5) hr.; foxglove aphid, 0.5-3.9 (1.7) hr.; lily aphid, 0.5-3.8 (1.5) hr.; celery aphid, 0.6-4.2 (1.5) hr. The values for the test feeding period were: green peach aphid, 0.1-4.5 (1.8) hr.; foxglove aphid, 0.3-3.7 (1.6) hr.; lily aphid, 0.3-3.9 (1.6) hr.; celery aphid, 0.4-4.3 (1.7) hr.

TABLE 4. *Results*[a] *of trials to determine effect of post-infection feeding starvation on vector efficiency with beet-mosaic virus. Single aphid apterae of 4 species were used. Infection feeding period varied from 10 to 29 sec. The preliminary starvation period has a mean value of approximately 1.5 hr., and the test feeding period had a mean value of approximately 1.6 hr.*

Species	Post-infection feeding starvation in min.							
	0	5	10	15	30	60	240	Total
Myzus persicae	8	3	1	0	0	0	0	12
M. solani	7	4	3	4	1	0	0	19
M. circumflexus	2	1	1	0	0	0[b]	0	4
Aphis apii	2	1[b]	0	1	0	0	0	4
Total	19	9	5	5	1	1	0	39

[a] Numbers in columns are number of plants infected out of 30 inoculated.

[b] One plant in series died, so number is that infected out of 29.

The virus was lost by all species during a relatively short period of starvation following an infection feeding (Table 4). Reduction was evident at the end of a 5-min. starvation period while complete loss occurred between the 30- and 60-min. intervals. The greatest and most rapid loss was found in the green peach aphid, since no transmission occurred after the 10-min. interval. However, retention for longer periods is possible (3). The foxglove aphid showed a slower rate of loss, infections being obtained up to and including the 30-min. interval, but no infection occurred with this or any species after that time. Transmission by the lily and the celery aphids was low and no transmissions were obtained after the 15-min. interval.

SUMMARY AND CONCLUSIONS

Experiments were made to determine the acquisition and inoculation thresholds and the effects of pre- and post-infection feeding starvation on transmission of beet-mosaic virus by the green peach aphid, *Myzus persicae* (Sulzer), the foxglove aphid, *M. solani* (Kalt.), the crescent marked lily aphid, *M. circumflexus* (Buckt.) and the celery aphid, *Aphis apii*, Theob.

None of the species was able either to pick up or to inoculate virus in a 5-sec. feeding period. The green peach and foxglove aphids were able to pick up and to inoculate virus in a 10-sec. period. The other two species required a 15-sec. period of feeding either to acquire or to inoculate virus. The green peach and the foxglove aphids were more efficient at acquiring or inoculating virus as the length of the feeding period was increased. An optimum value for acquisition, in the range tested (5 to 30 sec.), was obtained at the 20 to 25 sec. intervals. The inoculation threshold range tested (5 to 30 sec.) was too limited to secure an optimum value. Data on the lily and celery aphids were too limited to permit generalized conclusions, except to establish minimal acquisition and inoculation periods.

A pre-infection feeding starvation period of 5 min. was of benefit in increasing the efficiency of transmission by the green peach and foxglove aphids, with an optimum at the 60-min. interval. No conclusions were drawn as to the effect of preliminary starvation on transmission by the lily or celery aphids.

A post-infection feeding starvation period of 5 min. reduced efficiency of transmission of the green peach and foxglove aphids. With the former, no transmission occurred after a 10-min. interval. The foxglove aphid transmitted the virus up to and including a 30-min. interval, but failed thereafter. The lily aphid retained the virus for 10 min., the celery aphid for 15 min., but the data were too few to be of particular comparative value.

Factors responsible for varying degrees of efficiency of transmission of beet-mosaic virus by various species of aphids are still unknown. Differences among species seem to be in degree rather than in kind.

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PHYSIOLOGIC RACES OF THE FUSARIA CAUSING WILTS OF THE CRUCIFERAE [1]

G. M. Armstrong and Joanne K. Armstrong [2]

Fusarium conglutinans Wr. was first described as the cause of a yellows or wilt disease of cabbage, *Brassica oleracea* var. *capitata* L., but nearly all cultivated members of the cabbage tribe (cabbage, cauliflower, broccoli, brussels sprouts, kohlrabi, kale, and collards) have been found to be more or less susceptible (4, 8, 12). Recently Fusarium wilts of other Cruciferae have been noted, and the fungi named according to the system of Snyder and Hansen (11). The disease on radish (*Raphanus sativus* L.) was first reported by Kendrick and Snyder (5), and the causal fungus described as *Fusarium oxysporum* f. *raphani* n.f. Baker (2) established the fungus causing wilt of garden stock (*Mathiola incana* R.B.) as *Fusarium oxysporum* f. *mathioli* n.f. In an abstract in which no host varieties are given, Snyder (10) stated that occasionally cross infections among these 3 vascular *Fusaria* were possible, but each pathogen showed a highly selective specificity for its own host, thus warranting their distinction as separate physiologic forms of *Fusarium oxysporum* Schl.

The host relationships of numerous *Fusaria* of the section Elegans have been investigated by the writers for several years. After cabbage-wilt fungus was also found to cause wilting of radish, cultures of the radish-wilt *Fusarium* were obtained in 1949 from diseased radishes collected in Wisconsin[3] since radish wilt has not been reported in South Carolina. Isolates from wilted cabbage and radish were used to inoculate plants of varieties of the cabbage tribe, stock, and radish, as well as wilt-susceptible plants of other genera. Wilt *Fusaria* from plants of most of the other genera were also used to inoculate cabbage and radish. The main purpose of this investigation was to determine host relationships of *Fusarium* isolates from cabbage and radish. Classification and nomenclature of isolates are considered on the basis of these results.

Experimental Methods.—Plants were grown in 2-gallon pots of steamed sand and inoculated as previously described (1) by cutting some roots and pouring a liquid inoculum around them. For stock and members of the cabbage tribe, inoculations were made about 1 month, and for radish 15-17 days, after seed were sown. Plants were removed from pots when external symptoms of wilt were severe; otherwise they remained for 2 months after inoculation, the duration of the experiment. Experiments extended over a period of 3 years during fall, winter, and spring in a greenhouse with temperature maintained at approximately 28°C. Inoculations were made with isolates derived from single spores, but for comparison, mass isolates of the cabbage and radish *Fusaria* were occasionally used. Noninoculated plants of each species or variety grown concurrently with inoculated ones showed no external or internal symptoms of wilt.

Discussion and Results.—*Inoculation of Radish, Cabbage, Brussels Sprouts, and Kale with Wilt Fusaria from other Hosts.*—Long White Icicle radish and Copenhagen Market cabbage were inoculated with wilt *Fusaria* from the following hosts: alfalfa; bean; Cassia; cotton from India, from Egypt, and from the U.S.A.; cowpea (races 1 and 2); mimosa; muskmelon; English pea (race 2); sesame; spinach; sumac; sweet potato; flue-cured tobacco; tomato (race 1) and watermelon. One pot of 14 plants of radish and one pot of 5 or 6 plants of cabbage were used for each *Fusarium*. Eighteen plants of Improved Dwarf brussels sprouts were inoculated with a cotton-wilt *Fusarium* from the U.S.A. and 22 plants of Dwarf Siberian kale with an Egyptian cotton-wilt *Fusarium*. No external or internal symptoms of wilt appeared in any plants in these experiments.

Inoculation of Plants Other than the Cruciferae with the Cabbage and Radish wilt Fusaria.—Radish and cabbage *Fusaria* were used separately to inoculate plants of Grimm alfalfa, Mexican Pink bean, Hale's Best cantaloupe, *Cassia tora* L., Rowden cotton, California Blackeye 5 and Lady Finger cowpeas, bitter blue lupine, mimosa (*Albizzia julibrissin* Duraz.), Clemson Spineless okra, Thomas Laxton pea, Porto Rico sweet potato, Criollo sesame, Yelredo soybean, Bloomsdale Savoy spinach, sumac (*Rhus typhina* L.), Burley and Gold Dollar tobacco, Bonny Best tomato, and Watson watermelon. One pot of plants of each variety was used in the inoculations, with 5 or 6 plants per pot of tobacco, tomato, watermelon, and sweet potato, and 10 or more for all the others. No external or internal symptoms of wilt appeared in any plant.

Results of the preceding cross inoculations failed to reveal a relationship between the *Fusaria* of cabbage and radish and any of the 18 other *Fusaria* that were tested, since a common host was not found.

Inoculations of Stock, Radish, and Members of the Cabbage Tribe with the Cabbage and Radish Wilt Fusaria.—Results of inoculations of members of the cab-

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[2] Head, Department of Botany and Bacteriology, and Agent, Division of Cotton and Other Fiber Crops and Disease, respectively.

[3] Courtesy of A. R. Albert.

TABLE 1.—*Results of inoculations with the radish- and the cabbage-wilt Fusaria.*

Host [a]	Radish wilt Fusarium					Cabbage wilt Fusarium				
	Plants with wilt symptoms					Plants with wilt symptoms				
	Total external	Severe external	Slight external	Internal only	None	Total external	Severe external	Slight external	Internal only	None
	Per cent	Number	Number	Number	Number	Per cent	Number	Number	Number	Number
Brussels Sprouts:										
Improved Dwarf	19.6	16	4	55	27	96.0	24	0	1	0
Long Island Improved	61.3	40	9	27	4	96.4	27	0	1	0
Kale:										
Dwarf Siberian	1.0	2	0	118	78	55.1	7	20	21	1
Blue Curled Scotch	18.1	10	3	54	5	90.9	10	0	0	1
Early Green Curled Scotch	45.0	18	9	17	16	100.0	13	0	0	0
Stock:										
Giant Imperial Rose	0	0	0	9	0	50.0	4	0	4	0
Double Giant Shasta	90.0	6	3	1	0	0	0	0	5	5
Cabbage:										
Copenhagen Market	7.4	4	1	16	47	95.3	41	0	1	1
Danish Ballhead	0	0	0	8	11	100.0	12	0	0	0
Early Jersey Wakefield	0	0	0	1	3	87.5	7	0	1	0
Glory of Enkhuizen	0	0	0	2	16	–	–	–	–	–
Wisconsin All Season Resistant	0	0	0	0	8	30.0	2	1	1	6
Cauliflower: Early Snowball	2.6	2	0	58	17	88.2	15	0	0	2
Collards: Georgia	0	0	0	0	6	33.3	4	0	4	4
Kohlrabi: Green Vienna	0	0	0	0	7	26.7	4	0	3	8
Radish:										
Long White Icicle	97.5	73	6	1	1	87.5	95	3	5	9
Early Scarlet Globe	80.0	12	0	0	3	85.6	111	2	7	12
Round Black Spanish	–	–	–	–	–	72.0	79	6	22	11
French Breakfast	–	–	–	–	–	69.0	67	2	10	21

[a] Commercial lots of seed were used.

bage tribe, stock, and radish with isolates of a cabbage-wilt *Fusarium* from South Carolina and a radish-wilt *Fusarium* from Wisconsin are in Table 1. The cabbage *Fusarium* caused wilting not only of cabbage and other members of the cabbage tribe, but also of radish and of 1 variety of stock. The radish *Fusarium* caused wilting of radish, kale, brussels sprouts, and stock, but with a marked difference in susceptibility among the varieties of the last 3 hosts. Wilting occurred with only 1 variety of cabbage (7.4 per cent), although infection, as shown by internal symptoms, was present in many plants. The stock *Fusarium* was not available for comparison.

Comparison of results herein presented with those of others (2, 4, 8, 10) shows some differences which may be due to several factors: inoculation of a single variety with a wilt fungus may not delimit its potentialities as a pathogen for that crop (Table 1); isolates of these *Fusaria* from different regions may be different races, although Blank's (3) comparison of 19 isolates of *F. conglutinans* from 11 states indicates that this organism is uniform in its pathogenicity for cabbage and 6 other subspecies of *B. oleracea*. Comparison of radish isolates from various regions would also be desirable.

Padwick (6) pointed out that it is impossible to distinguish members of the sub-section Orthocera on cultural and morphological characters, proposing *F. orthoceras* var. *conglutinans* as the name for the cabbage yellows organism. Snyder and Hansen (11) have shown that even the criteria for distinguishing subsections of section Elegans are invalid, and proposed that all members of the section be called *F. oxysporum* with form names to designate the selective pathogenicity of each pathogen. In this system, *F. conglutinans* is *F. oxysporum* f. *conglutinans;* the radish fungus, *F. oxysporum* f. *raphani;* and the stock fungus, *F. oxysporum* f. *mathioli*. Padwick (7) stated that even the conception of the sections Elegans, Lateritium, and Liseola has so altered that they are no longer entirely distinct. He is opposed to the mass revision of Snyder and Hansen but concludes that their work must eventually influence classification within the genus.

When Snyder and Hansen revised the section Elegans, wilt *Fusaria* were believed to be so highly selective in pathogenicity that the method of designating the pathogens as forms of 1 species seemed to offer advantages of greater simplicity and usability. Some wilt *Fusaria* seem to be very specific in pathogenicity; however, an overlapping of hosts has been reported for certain forms. From investigations in progress there are indications that this also occurs with several others. Thus confusion arises in naming *Fusaria* from cabbage and radish according to this revised system. For example, if one obtained a wilt isolate from radish (Table 1), unaware that the plant had been inoculated with the cabbage *Fusarium*, and proved its pathogenicity on radish, the conclusion would be that

it is form *raphani*. Its pathogenicity to susceptible varieties of cabbage would make it form *conglutinans*, and to Giant Imperial Rose stock, form *mathioli*.

Since physiologic races have been accepted by many workers, and it seems evident that the cabbage, radish, and probably the stock *Fusaria* are races of *F. conglutinans* (13, 14), this system of designating the organisms should be used. Although a stock *Fusarium* was not available for comparison, wilting of some varieties of stock by cabbage and radish isolates in these tests, suggests that this may be a common host for establishing the race relationship of these *Fusaria*. Therefore, it is proposed that the host list for *F. conglutinans* be extended to include garden cress, mustard, radish, stock, and wallflower, and races 1, 2, and 3 to be used to designate specializations in its pathogenicity. Race 1 is most pathogenic to various members of the cabbage tribe, equally as pathogenic to radish as race 2, and causes wilting of at least 1 variety of stock. Race 2 is most pathogenic to radishes, less so or nonpathogenic to some members of the cabbage tribe which are susceptible to race 1, and also very pathogenic to at least 1 variety of stock. *F. oxysporum* f. *mathioli* is probably race 3 since Baker (2) found it did not cause wilt of the variety of cabbage, kale, and radish used in his tests. It is possible to differentiate races 1 and 2 by inoculating 1 variety each of cabbage and of radish, as for example, Early Jersey Wakefield cabbage and Early Scarlet Globe radish (Table 1). Further cross inoculations with *Fusaria* from stock, cabbage, and radish are needed to define race 3.

Although the writers agree with Snyder and Hansen (11) that cultural and morphological characters are not satisfactory criteria to distinguish the numerous wilt *Fusaria*, it is their opinion that not enough is now known about the host relationships of the numerous wilt *Fusaria* to attempt a mass revision on this basis. There seems to be no point in designating some as forms of one species if the system cannot be used for all. Therefore, with the section Elegans, use of species and variety names that are well established in the literature would seem to be the preferable system of nomenclature. Sherbakoff (9) has recently expressed a similar opinion.

SUMMARY

Plants of various members of the cabbage tribe, radish, and stock were inoculated with single spore isolates of wilt *Fusaria* from cabbage and radish. The cabbage *Fusarium* was highly pathogenic to radish, cabbage, and some other members of the cabbage tribe, and moderately so to one variety of stock.

The radish *Fusarium* was highly pathogenic to radish and one variety of stock, moderately so to a variety of brussels sprouts and kale, and less so or nonpathogenic to other members of the cabbage tribe. The cabbage and radish *Fusaria* produced no wilting of wilt-susceptible plants of 18 genera not in the Cruciferae. Inoculation of 1 variety each of cabbage and radish with 18 different wilt *Fusaria* from other hosts failed to produce any symptoms of wilt.

It is proposed that wilt *Fusaria* from cabbage, radish, and probably stock be designated as 3 physiologic races of *F. conglutinans* and not forms of *F. oxysporum*. There are several common hosts for cabbage and radish races, and it appears that stock is probably a common host for all 3. The fungus from cabbage is designated as race 1, and one with a high degree of pathogenicity chiefly for radish as race 2, and the one from stock probably race 3.

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TURNIP MOSAIC [1]

G. H. Berkeley and M. Weintraub

In 1946 a mosaic disease of rutabaga, *Brassica napobrassica* var. Laurentian, was observed near Walkerton, Ontario. At that time the virus nature of the disease was established by inoculation to turnip, *Nicotiana tabacum*, and *N. glutinosa*. Since then the disease has spread to other turnip-growing areas and has reduced yields considerably on certain farms, especially in the Walkerton district. The present paper deals mainly with symptomatology and identification of the virus, which was readily transmitted by the juice transfer method using carborundum.

LITERATURE.—Virus diseases have been reported on the following crucifers: turnips (2,3,4,13), cabbage (5,8,9,11,16), horseradish (7), cauliflower (10), stock (14), radish (15), and broccoli (1).

Several viruses concerned have a wide overlapping host range within the Cruciferae; certain ones infect plants outside this family. Paucity of information makes classification difficult. Recently Pound and Walker (8) and Walker, Le Beau and Pound (16) have shown that cabbage virus A and the cabbage black ring virus are strains of *turnip virus* 1 and that cabbage virus B is a strain of *cauliflower virus* 1. They also consider ring necrosis virus of cabbage (6) a strain of *turnip virus* 1 and the virus causing mosaic of broccoli in southern England (1) to belong to the *cauliflower virus* 1 group. Recently Larson, Matthews and Walker (16) have placed cabbage black ring, cabbage virus A, cabbage black ring spot (9) and horseradish mosaic (7) viruses as strains of *turnip virus* 1, and cabbage virus B and chinese cabbage mosaic virus as strains of *cauliflower virus* 1.

Symptomatology and host range under greenhouse conditions.—

N. tabacum—Only primary symptoms, consisting of necrotic local lesions with dark borders, developed on this host.

N. glutinosa—Symptoms consisted of chlorotic or necrotic primary lesions, followed by systemic chlorotic spotting with necrosis developing in the margins of chlorotic areas. Infected plants were severely stunted and lower leaves became necrotic but plants were not killed, except occasionally when very young plants were inoculated.

Petunia hybrida—Chlorotic primary lesions formed on rubbed leaves. Systemic symptoms consisted of chlorotic spot mottle, vein banding, and slight distortion of unfolding leaves.

Brassica napobrassica var. Laurentian turnip—Symptoms consisted of chlorotic primary lesions—sometimes lacking—followed by systemic clearing of veins, veinbanding, mottling, distortion of leaves, and ultimately small necrotic spots. Lower leaves were cast, and plants stunted severely. In the field, plants infected early in the season produced storage roots only 2 in. to 3 in. in diameter.

B. Pe-Tsai (chinese cabbage)—Chlorotic primary lesions generally developed on young plants but were sometimes lacking on older plants. Systemic symptoms consisted of mild general mottling with vein clearing on unfolding leaves. Innermost leaves especially tended to be unusually erect, with wavy margins.

B. oleracea var. *capitata* (cabbage var. Penn. State Ballhead)—Primary chlorotic spots or rings were few to none and systemic mottling was generally mild, with no distortion and only slight stunting of plants. Necrotic rings or solid necrotic spots were absent.

B. oleracea var. *botrytis* (cauliflower var. Perfected Snowball)—Primary chlorotic areas were rather indefinite and indistinct followed by mild systemic mottling.

B. napus (rape)—Fairly large, indefinite, chlorotic areas were present on rubbed leaves; systemic infection appeared as vein clearing, vein banding and general mosaic mottling, with curling of leaf margins.

Raphanus sativus (radish var. Scarlet Globe)—No symptoms developed, and transfers made from inoculated plants back to tobacco were negative, indicating that radish is not susceptible.

Sinapsis alba (mustard)—The first symptom was twisting, curling and distortion of unfolding centre leaves, with chlorotic areas on older leaves. Later, veinbanding and general mottle developed along with mild necrotic etching and distortion of new growth. Plants were stunted.

Matthiola incana (stocks)—Tan primary rings developed on rubbed leaves, followed by systemic, fine, greyish spotting with minute rings.

Lepidium sativum (peppergrass)—Prominent chlorotic lesions developed on rubbed leaves, followed by severe distortion, stunting, curling and twisting of unfolding centre leaves. Plants became yellow and ceased to grow.

Cheiranthus cheiri (wallflower)—Primary symptoms consisting of chlorotic and/or necrotic spots were followed by systemic mottling and slight distortion of unfolding leaves.

Capsella bursa-pastoris (shepherd's purse)—Chlorotic lesions developed on rubbed leaves, which later turned yellow and died. Systemic symptoms consisted of mottling, general yellowing and death of plants.

Partial Purification of the Virus.—Leaves of turnip, showing severe mosaic symptoms, were ground in a meat-grinder after removal of mid-ribs. Juice was extracted from the macerate by squeezing through broadcloth. Although the solid residue undoubtedly contained some virus, no attempt was made to recover this portion, and the residue was discarded. The juice, dark green in color, was heated to 40°C. for 10 min. then centrifuged at 3600 r.p.m. for 15 min. Ammonium sulphate was added to the resulting dark brown supernatant, at the rate of 25 gm. per 100 ml. of liquid. This solution was allowed to stand for 1 hr., then centrifuged for 20 min. (3600 r.p.m.). The precipitate of this centrifugation was taken up in water (1/5 of the original volume) and adjusted to pH 6.6 with N/10 NaOH.

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Since a preliminary test had shown that the supernatant of the centrifugation was still infective, this fraction was treated once more with 1/4 of its weight of ammonium sulphate, and centrifuged. The precipitate of the latter centrifugation was taken up in water, adjusted to pH 6.6, and the two precipitates were mixed together. This mixture was centrifuged, and the precipitate discarded, after it was shown to be non-infective.

In order to determine the isoelectric point of the virus, the resultant supernatant was subjected, in a preliminary test, to a range from pH 6.0 to pH 3.0. At each successive stage of increasing acidity, the mixture was centrifuged, and the precipitate tested for infectivity. It was found that the heaviest and most infective precipitate was obtained at pH 4.5, approximately the isoelectric point of the virus.

In subsequent trials, the supernatant was acidified directly to pH 4.5 with N/10 HCl. The solution was allowed to stand for 1 hour, after which it was centrifuged. The precipitate of this centrifugation was taken up in water, and dialysed against tap water for 24 hr. and against distilled water for 24 hr., at about 5°C. Contents of the dialysing sac were then centrifuged, after which the precipitate and supernatant were tested for infectivity. The final supernatant was a slightly opalescent liquid, faintly tinged with light brown. Both fractions possessed infective properties, and even after increasing length of dialysis, the final precipitate always appeared to retain as much infectivity as did the final supernatant.

Inclusion Bodies.—Turnip and *N. glutinosa* leaves were examined for inclusion bodies, using epidermal peelings stained with phloxine and trypan blue. Amoeboid-like bodies abounded but were restricted almost entirely to the subveinal epidermis (Fig. 1). Occasionally, one such body was found in cells of the interveinal epidermis. Size of these inclusion bodies varied, from 12μ x 6μ to 30μ x 12μ. They gave a positive test with Million's reagent, and, when dilute HCl was applied, became extremely coarse and granular. Such amoeboid bodies were not found in healthy tissues.

A number of irregularly-shaped crystals, of varying size, and soluble in HCl, were found in both healthy and diseased *N. glutinosa* leaves. In many mounts more crystals appeared in diseased than in healthy leaves.

Physical Properties.—Since the physical properties of a virus are of value in diagnosis, the thermal inactivation point, tolerance to dilution, and longevity in vitro of the turnip virus were ascertained. *Nicotiana tabacum* was used as the test plant.

The thermal inactivation point of the virus was 62°C. for a 10 min. exposure. Dilution end-point lay between 1-1000 and 1-10,000 while longevity at 22°C.–23°C. was between 24 and 36 hr.

When a purified extract of the virus was allowed to remain at room temperature for 22 hr., it lost its infectivity, whereas at 5°C. the purified extract was infective after 48 hr.

FIG. 1. Inclusion bodies in sub-veinal epidermal tissue of *N. glutinosa* leaves. Note 3 nuclei associated with inclusion bodies and 1 without. × 1100.

DISCUSSION.—Comparison of symptoms on a limited host range indicates that the Ontario turnip mosaic differs from the virus diseases of crucifers as reported in the literature. Absence of clearing of veins and prominence of necrotic ring-spotting on brassicas, especially cabbage, as reported by K. M. Smith (9) for ring spot, and by Tompkins, Gardner and Thomas (11) for black ring of cabbages, distinguish these diseases from the Ontario turnip mosaic. Ontario turnip virus also differs from chinese cabbage mosaic virus, reported by Tompkins and Thomas (12) to produce only primary symptoms on *N. glutinosa*. Thermal death point of their virus is approximately 13°C. higher than that of the Ontario virus. Lack of infection on rape, white mustard, wallflower and stocks by the turnip mosaic virus reported by Tompkins (13) varies from the results reported in this paper. The virus reported by Hoggan and Johnson (4) differs from the Ontario virus, not only in the symptoms it induces on *N. glutinosa*, but in thermal death point. The susceptibility of radish to cabbage viruses A and B (16) and to cauliflower mosaic virus (10) differentiates them from the Ontario turnip mosaic virus. Cauliflower mosaic virus (10) and cabbage virus B (16) are further differentiated by lack of infection on *N. tabacum* and *N. glutinosa*.

Although there are points of similarity between the

Ontario disease and the turnip mosaic reported by Clayton (3), the insusceptibility of cabbage to Clayton's virus suggests that the 2 are not identical. However, in view of the narrow range of hosts used by Clayton and the lack of information regarding the inactivation temperature, it is difficult to make significant comparisons. On the other hand, the Ontario virus and the turnip mosaic virus described by Chamberlain (2) in New Zealand, agree closely in host range, in symptoms on individual hosts, and in thermal inactivation temperatures. Both viruses failed to infect radish and both induced only primary lesions on tobacco as well as primary and systemic symptoms on *N. glutinosa*. The main difference between these 2 viruses is that the Ontario turnip virus produced primary lesions on turnip, chinese cabbage, cabbage, stock, wallflower, peppergrass, rape and shepherd's purse, whereas no primary symptoms are reported by Chamberlain on these hosts.

As stated by Larson, Matthews and Walker (6), previous work with crucifer viruses indicates that there are at least two groups: the turnip virus 1 group, including cabbage black ring, cabbage A, cabbage black ring spot, and horseradish mosaic viruses; and the cauliflower virus 1 group, comprising cauliflower mosaic, cabbage B and chinese cabbage viruses. This separation has been established on the basis of physical properties, cross protection, host range and serological reactions.

Comparison of physical characteristics of the Ontario turnip mosaic virus with those of the turnip virus 1 group and the cauliflower virus 1 group, as defined by Larson, Matthews and Walker (6), is shown in Table 1.

The foregoing data indicate that the Ontario turnip mosaic virus agrees more closely with the turnip virus 1 group than with the cauliflower virus 1 group, in thermal inactivation point, in dilution tolerance, and in host range. In spite of its divergence in aging *in vitro*, the Ontario turnip mosaic virus probably belongs in the turnip virus 1 group.

TABLE 1.—*Physical properties of certain crucifer viruses*

Virus	Aging in vitro (hr. at 20°C.)	Dilution Tolerance	Thermal Inactivation Point (°C. for 10 min.)
Turnip Virus 1 group	72 to 92	1-700 to 1-15,000	59 to 62
Cauliflower Virus 1 group	96 to 300	1-500 to 1-6,000	75 to 78
Ontario Turnip Mosaic Virus	24 to 36 (22-23°C.)	1-1,000 1-10,000	62

SUMMARY

Symptoms on 14 different hosts have been described for a virus causing a mosaic disease on turnips in Ontario, Canada.

The causal virus was partially purified by chemical means and centrifugation, and its isoelectric point found to be approximately pH 4.5. Inclusion bodies of the amoeboid type were plentiful only in sub-veinal tissues. Thermal death point of the virus was 62°C. for a 10 min. exposure; tolerance to dilution was between 1-1000 and 1-10,000; longevity *in vitro* at 22°C.–23°C. was between 24 and 36 hr.

Comparison of symptoms on a limited host range indicates that the Ontario turnip mosaic differs from the several virus diseases of crucifers as reported in the literature. The Ontario disease closely resembles that reported by Chamberlain in New Zealand, and probably belongs to the turnip virus 1 group.

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APHID TRANSMISSION OF A STRAIN OF ALFALFA MOSAIC VIRUS [1]

K. G. Swenson [2]

Host range, symptomatology, and physical properties of alfalfa mosaic viruses have received considerable attention, but relationships to insect vectors have not. The type strain has been transmitted by *Macrosiphum pisi* (7), the potato calico strain by *M. solanifolii* (4), and the potato necrosis strain by *Myzus persicae* (3). Kovachevsky transmitted an alfalfa mosaic virus to chili pepper with *M. persicae* (1). The present work deals with transmission ability of various species of aphids and transmission by melon and pea aphids.

MATERIALS AND METHODS.—The virus was obtained from Dr. J. W. Oswald who secured it from Ladino clover (variety of *Trifolium repens* L.) near Red Bluff, California. Symptoms produced on a series of host plants resembled in general those produced by the type strain of alfalfa mosaic virus (8). However, the strain from Ladino clover produced symptoms in *Zinnia elegans* Jacq. which the type strain did not (8). The Ladino clover strain is not identical with that described by Kreitlow and Price (2) since it produced much less severe symptoms on *Nicotiana tabacum* L. and infected alfalfa. Although not identical with Oswald's potato tuber-necrosis strain, it too produces tuber-necrosis in potato (3).

Aphids were obtained from non-infective stock colonies maintained in a greenhouse. Transfers were made with a camel's-hair brush. During starvation periods, aphids were kept in shell vials. During feeding periods of 1 min. or less, aphids were observed through a handlens and length of feeding was timed with a stopwatch. Feeding periods longer than 1 min. represent the time aphids were on the plants and not actual feeding time.

Pepper (*Capsicum frutescens* L.) was used both as a virus source plant and as a test plant in experiments with the melon aphid, *Aphis gossypii*. It was also used as a virus source plant for the pea aphid, *Macrosiphum pisi*, but broad bean (*Vicia faba* L.) was used as a test plant. To determine the species capable of transmitting alfalfa mosaic virus, aphids remained on test plants for 3 days. In other tests they were left on the test plant for 4 hr. Plants were then fumigated in a nicotine chamber and placed in an insect-free greenhouse.

VECTORS.—Transmission tests were conducted with 12 species of aphids to determine most efficient vectors and species able to transmit the virus. The 12 species include aphids commonly found to transmit viruses and also species commonly found on leguminous plants.

Celery was used both as virus source plant and as a test plant for *Aphis apii*, *A. ferruginea-striata*, and *Cavariella aegopodii*. For the other 9 species, the virus source plant was either pepper or jimsonweed, except that for *Aphis fabae* broadbean was used, and the test plant was pepper or broadbean. Twenty aphids were fed on each test plant for 3 days.

TABLE 1.—*Transmission of alfalfa mosaic virus by various species of aphids*

Aphis apii Theob.	0/20[a]
A. ferruginea-striata Essig	0/20
A. gossypii Glover	13/25
A. medicaginis Koch	1/10
A. fabae Scop.	2/20
Cavariella aegopodii (Scop.)	0/20
Macrosiphum pisi (Kalt.)	9/20
M. solanifolii (Ash.)	3/10
Myzus circumflexus (Buckt.)	0/20
M. ornatus Laing	0/20
M. persicae (Sulz.)	5/20
M. solani (Kalt.)	0/20

[a] Denominator, number of test plants used; numerator, number of plants infected.

TYPE OF VECTOR RELATIONSHIP.—Watson and Roberts (6) divided the insect-transmitted plant viruses into two groups, persistent and non-persistent, insofar as relationships with their insect vectors are concerned. Non-persistent viruses respond, with a markedly increased amount of transmission, to a period of starvation immediately preceding the feeding period on the diseased plant, provided that the feeding period on the diseased plant, the infection feeding, is quite short (a few minutes or less). A starvation period does not increase amount of transmission by vectors of persistent viruses. Non-persistent viruses are retained for a few hours, or less, by their vectors, whereas persistent viruses are retained for a considerable length of time, often as long as the insect lives. Moreover, non-persistent viruses require no latent period in the vector before transmission to a healthy plant can occur, as is generally required by persistent viruses, but can be transmitted immediately after feeding on a diseased plant even though the feeding period there may have been only 10 sec. (5).

Using these differences as criteria, experiments were conducted to determine the group to which alfalfa

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The writer is indebted to Professor E. O. Essig for determination of *Aphis medicaginis*, and to Dr. E. S. Sylvester for aid in procuring several of the aphid species used.

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mosaic virus belongs. Of the 2 most efficient vectors, the melon aphid was used in preference to the pea aphid because it was easier to handle and gave somewhat better transmission, although the pea aphid is more often found in nature feeding on plants naturally susceptible to alfalfa mosaic virus.

Response to starvation.—In these tests, melon aphids were starved for 1 hr. before the feeding period on the diseased plant and then, without further starvation, transferred, 5 to each plant, to 25 healthy pepper plants (Table 2). Another group of aphids was treated in the same manner except that they were not starved before feeding on the diseased plant (Table 2). Both groups fed for 5 min. on the diseased plant.

TABLE 2.—*Transmission of alfalfa mosaic virus by melon aphids starved immediately before and after a feeding period on a diseased plant*

Starvation before	None	1 hr.		
Starvation after	None	None	1 hr.	4 hr.
	5/25[a]	10/25	0/25	0/25

[a] Denominator, number of test plants used; numerator, number of plants infected.

Although a greatly increased amount of transmission can be obtained by a period of fasting preliminary to the infection feeding period, this effect characteristically decreases with increasing length of the infection feeding period. Table 3 shows transmissions obtained with single aphids subjected to various infection feeding periods after a preliminary starvation period of ½ hr.

TABLE 3:—*Transmission of alfalfa mosaic virus by single melon aphids subjected to various infection feeding periods following a starvation period of 30 minutes*

Infection feeding period (in min.)			
1	10	30	60
11/40[a]	4/40	1/40	1/40

[a] Denominator, number of test plants used; numerator, number of plants infected.

Non-retention of virus in the vector.—Two groups of aphids that had been starved for 1 hr. before an infection feeding of 5 min. were removed from the diseased plant and placed in shell vials. One group was left in the shell vials for 1 hr. and the aphids were then placed, 5 to a plant, on 25 healthy pepper plants (Table 2). The other group was removed at the end of 4 hr. and similarly disposed (Table 2). Neither group of aphids transmitted the virus, indicating that alfalfa mosaic virus was retained for less than an hour under these conditions.

Absence of latent period.—In one series of tests, 30 pea aphids were starved for 1 hr. and placed on the virus source plant for an infection feeding of 15-45 sec. They were then removed and immediately placed, singly, on healthy broadbean plants where they were allowed to feed for periods varying from 10-20 sec. The virus was transmitted to 6 of the 30 plants. Since the total time spent on both the diseased plant and the healthy test plant varied with individual aphids from not less than 25 sec. to not more than 65 sec., it is apparent that no latent period was involved.

SUMMARY

Alfalfa mosaic virus was transmitted most efficiently by *Aphis gossypii* and *Macrosiphum pisi*. It was also transmitted by *Aphis medicaginis*, *A. fabae*, *Macrosiphum solanifolii*, and *Myzus persicae*. Six other aphid species failed to transmit it.

Alfalfa mosaic virus was shown to belong to the non-persistent group of insect-transmitted plant viruses since it exhibited the definitive characteristics of that group. A period of starvation of the melon aphid immediately preceding a short feeding period on a diseased plant greatly increased the amount of transmission over that obtained without such a period of starvation. The virus was retained by non-feeding aphids for less than 1 hr. and was transmitted without a latent period in the vector.

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PRESERVATION OF PUCCINIA UREDOSPORES BY LYOPHILIZATION [1]

Eugene L. Sharp and Frederick G. Smith

Maintenance of *Puccinia* collections is a constant problem for the cereal pathologist and breeder. By previous methods of storage, mainly refrigeration of uredospores or infected leaves, viable inoculum has seldom been preserved for more than a few months to a year (3, 4, 8). It has been necessary, therefore, to maintain the organism by periodic passage through a host plant. This not only is laborious but involves risk of contamination and mutation. Furthermore, exchange of *Puccinia* collections among widely separated laboratories has been hazardous. For these reasons it would be highly desirable to have a means of preserving type collections for periods of several years in a form excluding mutation and stable enough to allow shipment.

The lyophilization technic widely used for bacteria (2) and successfully applied to a number of fungi by Raper's group (1, 7) offered the best possibility. An apparatus similar to that of Raper and Alexander (7) was used, with their conditions of processing serving as model. Most of the present work was done with *Puccinia coronata* Corda, the oat crown rust pathogen. For a more accurate measure of survival, percentage germination was determined rather than infectivity alone. Petri dishes containing 3 percent gelatin provided a substrate of proper consistency and moisture conditions and gave an optical clarity suitable for germination counts. Spores were dusted on the surface with a camel's hair brush. Under these conditions, designated Germination Method A, fresh collections of spores regularly germinated 95 to 100 per cent.

Lyophilization of spores with the commonly-used suspending media, blood serum, gelatin or sucrose solutions, gave no measurable survival. Without suspending media, however, the method showed promise and the effects of time and temperature of drying and vacuum were investigated. Two main types of lyophilization were used. In Lyophil Method F (modelled after Raper and Alexander (7)) spores were first frozen at —45 to —50° C. and then dried under vacuums ranging from 20 to 150 microns. The temperature was raised to about —10° C. during the first drying period of 2 to 3 hours and then to room temperature during the second period of about 1 hour. In Lyophil Method NF no initial freeze was used and the lyophil tubes were kept at room temperature throughout a 3 hour drying period.

With Lyophil Method F the time and temperature of the drying periods were found to affect the per cent survival both during processing and storage. Survival during processing ranged from 50 to 75 per cent, depending chiefly upon the age of the collection, and the germination then remained fairly constant for some weeks before declining slowly to less than 1 per cent. However, these apparently non-viable spores were found to be still infective and the per cent germination was raised to the original post-lyophil level (Fig. 1) if the dried spores were exposed at room temperature in a saturated moist chamber for 24 hours before being dusted on the gelatin. These conditions are designated Germination Method B. Apparently spores subjected to this type of lyophilization and storage must be rehydrated more slowly than occurs in the water film of the gelatin (i.e., in Germination Method A). Lyophil Method NF gave somewhat higher survival during processing, 85 to 90 per cent, especially with fresh collections and dried spores were less sensitive to conditions of germination. Non-lyophilized spores showed no difference in per cent germination between Methods A and B.

Under optimum conditions both methods of lyophilization have given excellent survival of *P. coronata* and *P. graminis* (*avenae*) for periods now up to about 500 and 300 days, respectively (Table 1). Within the limits of accuracy of the viability assay there was no evidence of change in viability during storage of *P. coronata* spores. Further work will be necessary, however, to determine whether processing method F or NF is definitely superior for long term storage. Non-lyophilized spores of the same collections were

TABLE 1. *Survival of lyophilized Puccinia uredospores (PC = P. coronata, PG = P. graminis)*

Collection	Lyophil Method	Storage time (days)	Percent survival[a] Non-lyo. spores	Lyophilized spores
PC I 5-30	F	311	0-1[b]	52
		529	0-1[b]	55
PC I 6-17b	F	292	0-1	52
PC II 7-3a	F	280	55	77
		495	19	77
PC II 8-5	F	247	62	65
		462	21	70
	NF	241	62	95
PC II 10-13	NF	173	66	85
		359	17	83
PC III 1-20	F	81	25	65
		284	0-1	56
PC IV 11-24 (race 57)	F	130	0-1	35
		224	0-1	35
PG I 5-30	F	309	0-1[b]	40
PG II 4-7-51	F	82	58	74
		210	0-1	69

[a] Based on per cent germination before lyophilizing. Differences greater than 8 per cent are significant at the 2 per cent level (5). Non-lyophilized, by Germination Method A; Lyophilized, by Germination Method B.

[b] Showed no infection on susceptible host.

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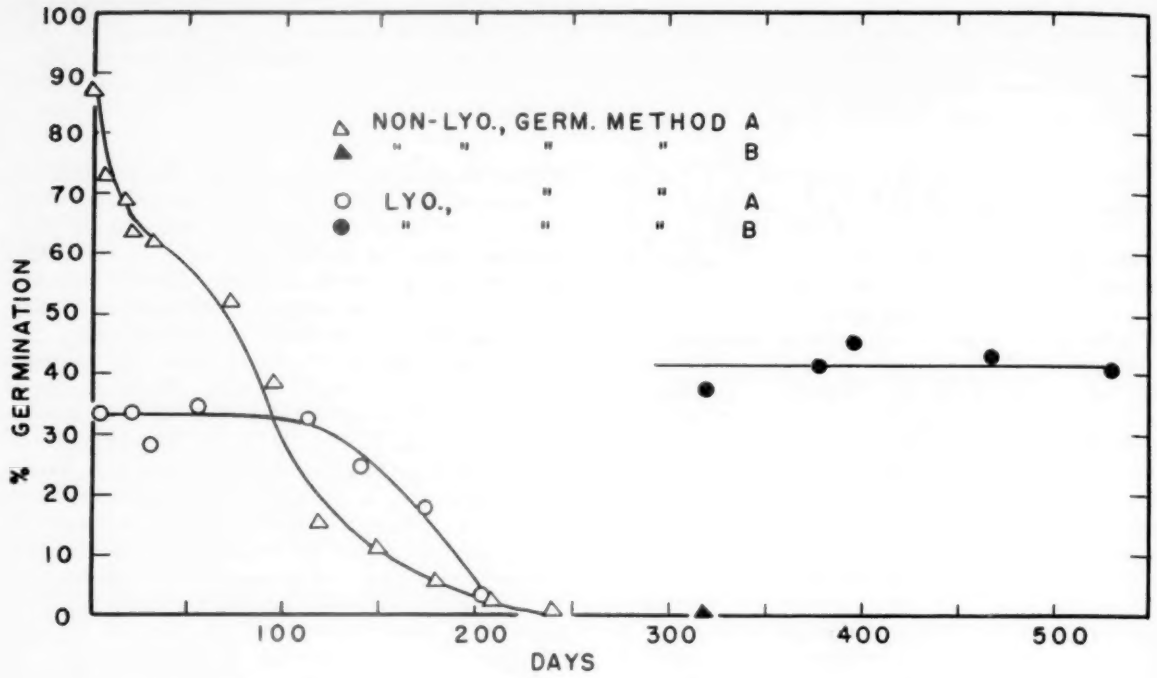

Fig. 1.— Comparison of survival of lyophilized and non-lyophilized uredospores of *P. coronata*.

stored in stoppered tubes at 5-10° C. and 25-50 per cent relative humidity. Their viability dropped markedly in all cases and was less than 1 per cent in most collections after a year. Only in the field collection of *P. coronata*, PC II, was there measurable survival of non-lyophilized spores after a year's storage.

Pathogenicity tests with representative lyophilized and non-lyophilized samples of *P. coronata* and *P. graminis* uredospores have shown satisfactory agreement between infection ratings (8) on Markton oats and per cent germination by Method B. The effect of lyophilization on mean type of infection rating was also tested on selected members of differential oat varieties (6) using pure race 88 and a mixture of races 45 and 57 of *P. coronata*. The lyophilized spores gave the same reactions as fresh spores indicating no physiological change during processing or storage. Limited results with pure races so far have shown no differences in response to lyophilization.

Lyophil tubes have been held at room temperature for several months without loss of spore viability indicating that cultures can be shipped in this form. The importance of maintaining the vacuum during storage was indicated by loss of viability in several tubes which developed small cracks during storage. Work so far has been chiefly with 5 to 10 mg. of spores per tube, but recent batches as large as 500 mg. have survived processing equally well and appear to be stable in storage.

The results so far indicate that *P. coronata* and *P. graminis* uredospores are readily preserved by lyophilization without change in pathogenicity. The excellent storage life of other fungus spores, which have survived lyophilization (1), indicates that *Puccinia* uredospores may be expected to remain viable for several years. This method would provide a basis for the establishment of permanent type culture collections of the many races and biotypes of *Puccinia* species and may offer a similar possibility with other obligate plant parasites.

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CACHEXIA DISEASE, ITS BUD TRANSMISSION AND RELATION TO XYLOPOROSIS AND TO TRISTEZA [1]

J. F. L. Childs [2]

In 1950 the author (2) reported that Cachexia, an apparently new disease affecting Orlando tangelo (*Citrus paridisi* x *C. reticulata*) trees on several different rootstocks in Florida, is characterized by stunting, vein chlorosis, gum impregnation and discoloration of the phloem (Fig. 1A), wood pitting (Fig. 1B), and bark cankers (Fig. 1C). Gum is also deposited in the wood in bands or segments of bands that follow the growth rings of the xylem. Similar symptoms have been found on other species and varieties of citrus and citrus relatives and it now appears that the disease is far more widespread than was at first suspected.

In preliminary observations it was shown that neither incompatibility between root and scion nor boron deficiency could have causal relation to the disease, and it was considered unlikely that a species of Phytophthora, Diplodia, or some other common fungus or bacterium was the cause. On the other hand, presence of psorosis-like leaf patterns on a number of affected Orlando tangelos in one grove and the absence of spread from tree to tree in the orchard both suggested a bud-transmitted pathogen as the causal agent. Accordingly, an experiment on bud transmission of the disease was undertaken in 1947 (2) and the results now available are presented in this paper.

METHODS.—One-year-old Rough lemon seedlings, 240 in number, were set out in lots of 30 each. These were budded in place as follows: (a) buds from Orlando tangelo trees affected with cachexia, as determined by phloem discoloration, were inserted in 3 lots of seedlings. The buds used in each lot came from a different grove and each seedling was budded from a separate diseased Orlando tangelo. (b) Buds from healthy Orlando tangelos, as determined by the absence of phloem discoloration and the generally vigorous appearance in trees 5 yr. or more of age, were inserted in 5 lots of seedlings. (c) One lot of Rough lemon stocks carrying healthy Orlando buds was budded at the same time with psorosis (type A) infected buds from a Valencia orange tree for the purpose of observing expression of psorosis leaf sypmtoms in Orlando tangelo. The Valencia buds were inserted in the Rough lemon stock below the Orlando bud. In the spring of 1948 each Rough lemon stock was cut back to the Orlando bud which was forced into growth to form the top of the tree (2).

In 1949 a number of grapefruit trees were found that had been top-worked to Orlando tangelo. Although the Orlando buds had come from healthy trees, a large proportion of the scions showed symptoms of cachexia above the union with the grapefruit. In 1950 grapefruit buds from these trees were inserted in another lot of healthy Orlando trees on Rough lemon rootstock.

Ninety Orlando tangelo seedlings, also set out in 1947, were budded as follows: 30 were budded with diseased Orlando buds, 30 with healthy Orlando buds, and 30 were left unbudded as checks. These trees were not cut back to the inserted bud.

RESULTS OF THE TRANSMISSION EXPERIMENT.—Inspection over a 2-yr. period revealed no psorosis-like leaf symptoms except in the lot of 30 trees in which the psorosis (type A) infected Valencia orange buds had been inserted (2), although all Orlando tangelo trees on Rough lemon stocks were cut back repeatedly, forcing additional flushes of foliage for the purpose of leaf symptom expression.

From time to time the outer bark of a few of the larger trees was carefully shaved off at the bud union to expose possible discoloration in the Orlando phloem tissues at the rootstock boundary. In February 1951 the first symptoms were found and all trees in the experiment were immediately examined. Discoloration of the phloem, above the bud union (Fig. 1) was found in the 3 lots of trees whose tops consisted of Orlando tangelo grown from diseased buds. Symptoms were expressed in 62, 69, and 94 per cent of the trees in the 3 lots. In October 1951 the trees were examined again and the number of trees showing symptoms was found to have increased to 95 and 87 per cent in the first 2 lots (Table 1) but no change was found in the third lot. Discoloration was not found in any scion in the 3 lots of trees budded from healthy Orlando trees in the February or in the October examination.

In the Orlando seedlings which were inoculated by inserting cachexia infected buds, phloem discoloration was found in 93 per cent of the trees in February. No increase was found in the October examination. Discoloration of the phloem in the Orlando stock was found to have spread much farther in a downward direction than upward from the point of insertion of the diseased bud. No phloem symptoms were found in trees resulting from healthy buds on Orlando stocks or in unbudded Orlando seedlings in either examination.

In the group of healthy Orlando tangelo on Rough lemon rootstock in which buds from the topworked grapefruit, referred to above, were inserted for inoculation, very few grapefruit buds survived because of unusually hot weather at the time of budding. However, 7.7 and 8.7 per cent of Orlando scions expressed phloem symptoms of cachexia in the first and second examinations, respectively.

A striking development occurred in trees in which both healthy Orlando buds and psorosis-infected Valen-

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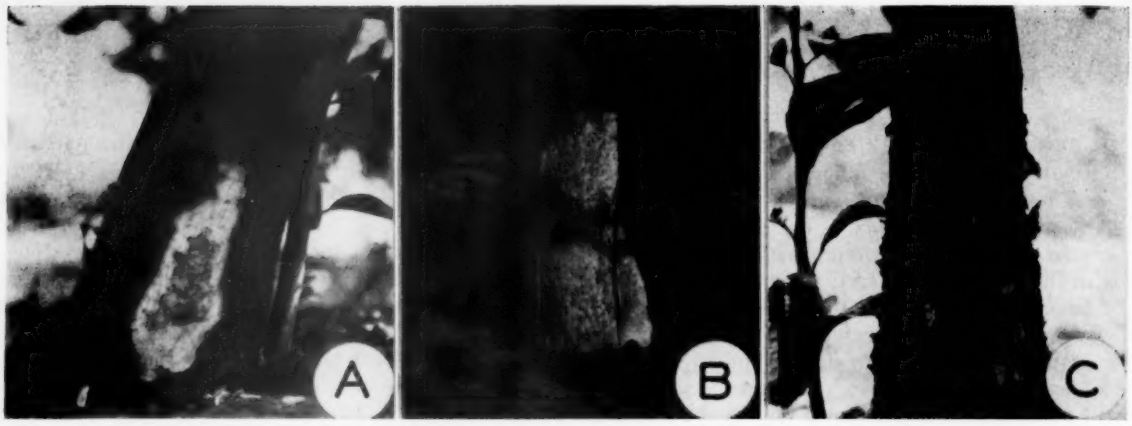

FIG. 1—A. Discoloration of the phloem of Orlando tangelo scion on Rough lemon stock due to impregnation with gum; B. Pitting of the wood of Orlando on Rough lemon stock. Note smooth surface of wood below the union boundary; C. Bark canker on Orlando scion. Cankers appear in the later stages of the disease.

cia orange buds had been inserted in the Rough lemon seedlings. Of the Orlando scions that survived, 72 per cent exhibited bark symptoms of cachexia in the first examination and 84 per cent showed symptoms in the second examination.

Cachexia symptoms in other varieties of citrus and citrus relatives. Although originally not intended to be part of the research on cachexia disease, the rootstock experiments set up in Florida and Texas in 1948 have yielded important information on the expression of cachexia symptoms by other varieties of citrus and citrus relatives and on symptomless carriers. These plantings consisted of seedlings of many different varieties of citrus and a few citrus relatives budded with 2 standard varieties of sweet organge (Valencia and Pope Summer) in Florida and a variety of grapefruit (Red Blush) in Texas. Although an effort was made to secure psorosis-free buds of Red Blush grapefruit, it was subsequently discovered on the basis of leaf symptoms that some bud sources were psorosis-infected.

TABLE 1.—*Transmission of cachexia disease by means of buds from infected Orlando trees budded on Rough lemon and on Orlando tangelos rootstock.*

Rootstock and Plot No.	Source of Orlando buds	No. of surviving Orlando scions	Per cent of scions with symptoms	
			Feb.[a]	Oct.
Rough lemon	Diseased			
Plot No. 1	Grove No. 1	21	61.9	95.3
Plot No. 2	Diseased			
	Grove No. 2	16	68.8	86.7
Plot No. 3	Diseased			
	Grove No. 3	17	94.2	94.2
Plot No. 4	Healthy			
	Grove No. 4	21	0	0
Plot No. 5	Healthy			
	Grove No. 1	26	0	0
Plot No. 6	Healthy			
	Grove No. 5	24	0	0
Plot No. 7	Healthy [b]			
	Grove No. 5	25	72.0	84.0
Plot No. 8	Healthy [c]			
Orlando tangelo	Grove No. 1	26	7.7	8.7
Plot No. 1	Diseased			
	Grove No. 2	27	92.6	92.6
Plot No. 2	Healthy			
	Grove No. 3	28	0	0
Plot No. 3	.....	30	0	0

[a] Buds were inserted during October 1947 and scions examined in 1951.

[b] Budded at the same time with psorosis A infected buds from Valencia orange.

[c] Buds from grapefruit interstock inserted in the Rough lemon stocks in July 1950.

In March 1951 E. O. Olson reported to the author that rootstocks of Orlando tangelo and Temple orange (probably a mandarin hybrid) in the Texas planting showed phloem discoloration and wood pitting symptoms of cachexia. Upon examination of the other rootstocks in the Texas planting he found 11 other citrus varieties and hybrids with cachexia symptoms (11). Examination of the Florida rootstock plots (3) in June 1951 revealed that pholem symptoms of cachexia were expressed by 10 of the 13 tangelo (*Citrus paradisi* x *C. reticulata*) varieties, by 3 of the 8 mandarin orange (*C. reticulata*) varieties, by the kumquat (*Fortunella margarita*) in the test, by the calamondin (*C. reticulata* x *Fortunella*), by the 2 limequats (*C. aurantifolia* x *F. japonica*), an by 2 of the tangors (*C. reticulata* x *C. sinensis*).

Phloem symptoms were not expressed in the following rootstocks budded with buds from the same sources: 2 varieties of sweet orange (*C. sinensis*), 6 varieties of sour orange (*C. aurantium*), 2 varieties of grapefruit (*C. paradisi*), 5 varieties of pummelo and shaddock (*C. grandis*), 2 varieties of *Poncirus trifoliata*, 2 varieties (probably hybrids) of lemon (*C. limon*), 2 lime (*C. aurantifolia*) hybrids, 5 varieties of citrange (*P. trifoliata* x *C. sinensis*), 3 varieties of citrumelo (*P. trifoliata* x *C. paradisi*), 2 varieties of citrangor ([*P. trifoliata* x *C. sinensis*] x *C. sinensis*), or in citrangequat ([*P. trifoliata* x *C. sinensis*] x *Fortunella*). It is evident that many buds used in the rootstock trials in Florida and Texas were carrying the causal agent of cachexia. This constitutes further evidence of the existence of symptomless carriers.

Relation of cachexia to xyloporosis.—The remarkable similarity between the symptoms of cachexia of Orland tangelo and the symptoms of xyloporosis of sweet lime root stocks (Shamouti sweet orange scions) in Palestine (12) and of sweet lime rootstocks (Barao sweet orange scions) in Brazil (9) suggested that these 2 diseases are closely related if not identical.

Attempts to obtain specimens of xyloporosis-affected wood from Palestine for purpose of direct comparison were unsuccessful but through the kindness of A. S. Costa, specimens preserved in alcohol-acetic-acid-formaldehyde solution were obtained from Brazil. These specimens were cut from Bahia navel orange on Lima da Persia (sweet lime) root in such a manner that the bud union bisected each specimen. The sweet lime portions showed typical pitting of the cambial surface and the annular deposits of gum on exposed surfaces of radial sections, as described by Reichert and Perlberger (12). These symptoms, together with certain additional aspects of the pathological anatomy not previously described, correspond in every detail to the disease recognized as cachexia on Orlando tangelo (2) and strongly suggest that these 2 diseases are identical.

That wood-pitting is reported only on sweet lime stocks in one instance and appears on Orlando tangelo stocks and scions in another is explainable as a tissue reaction symptomatic of the disease and peculiar to sweet lime, Orlando tangelo, and certain other citrus varieties and citrus relatives regardless of whether they are stocks or scions. Reichert and Perlberger (12) noted that xyloporosis had been found on sweet lime stocks grafted with sour lemon, grapefruit and mandarin and that the disease passed to the mandarin but not to the sour lemon and grapefruit scions. Data presented above confirm the susceptibility of some mandarin varieties and absence of symptoms on grapefruit and sweet orange. Reichert and Perlberger also reported xyloporosis symptoms on sweet lime seedlings (12), mainly on those that had been cut back, and noted that pitting was localized at the point of cutting. This strongly suggests that these trees had been budded with diseased buds and that the buds later died. When cachexia-infected Orlando tangelo buds were inserted in Orlando seedlings, symptoms were similarly localized in the stock at the point of bud insertion, in the early stages.

Reichert and Perlberger attributed xyloporosis to physiological factors (12). Moreira (9) attributed it to incompatibility between stock and scion, citing Barao sweet orange on sweet lime stock as an example, but noted that other sweet orange varieties (Caipera and Pera) were perfectly healthy on sweet lime stock. Reichert and Perlberger (13) maintained that incompatibility is a physiological factor that they had envisaged originally. Wallace (14) suggested that xyloporosis is a heritable condition that is probably accentuated by certain environmental factors, particularly stock-scion relationships.

The fact that cachexia has been transmitted from one host to another by means of buds suggests that wood pitting and other abnormalities are due to a transmissable agent and might be considered evidence that the causal agent is a virus. However, as Dimock (5) indicated, mere bud transmission is not proof of the virus nature of the causal agent. Moreover, certain aspects of the pathological anatomy of cachexia affected trees, which are being investigated, cause us to question the validity of the virus theory.

Relation to tristeza.—On the basis of wood-pitting symptoms, McClean (8) has suggested that xyloporosis disease on sweet lime is a form of the aphid-transmitted, stem-pitting disease of grapefruit in South Africa. On the basis of pitting alone there is a point of similarity between cachexia and the South Africa grapefruit disease (2, 10). However, Costa, Grant and Moreira (4) showed that, on the basis of insect transmission and expression of leaf symptoms, the wood-pitting disease of grapefruit and tristeza are the same or closely related, but they (7) distinguished between tristeza and xyloporosis on the basis of differential host reaction. On the basis of work performed in Brazil (9) and in Palestine (12), Wallace (14) also concluded that tristeza is distinct from xyloporosis.

Evidence obtained in the present investigation gives additional proof that cachexia (xyloporosis) and tristeza are different diseases. Studies on symptom expression (3) indicate that many mandarins are injured by cachexia as is indicated by gum impregnation of the phloem tissues, and in the case of the mandarin hybrids, such as the tangelos, that susceptibility seems to stem from the mandarin parent, but Grant, Costa and Moreira (6) found the mandarins to be extremely tolerant of the tristeza virus. Cachexia infected sweet orange buds from 2 sources in Florida and grapefruit buds from a source in Texas caused cachexia symptoms to be expressed on susceptible mandarins and tangelos, but buds from the same source trees grew vigorously on 6 different sour orange rootstocks, which is certainly contrary to the observed reaction of these varieties on sour orange root when infected with tristeza (1, 6).

Discussion and Conclusions.—The expression of symptoms on scions taken from diseased trees is regarded not as an instance of disease transmission but as an instance of perpetuation of the disease symptoms. Without question, the appearance of phloem discoloration on the Orlando seedlings budded with cachexia-infected Orlando buds (Table 1, Orlando stocks, plot No. 1) is evidence of disease transmission. The expression of symptoms in Orlando tangelos budded with buds from symptomless carriers (Table 1, grapefruit buds, and in rootstock plantings of Texas and Florida) is further evidence of transmission of cachexia disease.

That symptomless carriers of cachexia exist is shown by the expression of symptoms in Orlando tangelos that had been inoculated by buds from trees showing no phloem or bark symptoms of cachexia (plot 8, Table 1). Further examples appeared in our rootstock plantings in Florida and Texas. Two sweet orange varieties and the single grapefruit variety used

in these tests carried the cachexia disease agent as indicated by symptoms on Orlando tangelo and other varieties.

Occurrence of phloem-symptoms of cachexia in Orlando scions on Rough lemon rootstocks that were budded with psorosis-infected Valencia orange buds (Table 1, Rough lemon stock, Plot No. 7), presents a problem of interpretation. Three interpretations seem worthy of consideration. In the first, the psorosis (type A) infected Valencia orange tree may be regarded as a symptomless carrier (of the cachexia disease agent) in which all the disease symptoms are due to psorosis and none are due to cachexia. In the second, psorosis (type A) may be regarded as a dual disease induced in part by a virus that causes the exhibition of psorosis leaf symptoms and in part by the agent of a second disease (cachexia). In the third, cachexia may be regarded as identical with psorosis A. However, this last hypothesis seems untenable in view of the absence of psorosis leaf symptoms in all cachexia affected Orlando tangelos (table 1), except those inoculated with psorosis A. Further work on this phase is in progress.

Observation of rootstock material in Texas and Florida shows that discoloration of the phloem due to gum impregnation is readily expressed in some mandarin oranges (*C. reticulata*), in kumquat (*Fortunella margarita*), and in hybrids of mandarin and of *F. japonica*. Phloem discoloration is much more pronounced in some varieties than in others, indicating a range in severity of symptoms among different varieties examined. To date, only a few of the rootstocks have been examined for wood-pitting, so that the degree of wood-pitting in different citrus varieties and citrus relatives is not clear at this time. Wood-pitting and certain other definitive symptoms of cachexia have been found regularly in Orlando tangelo, sweet lemon (sweet lime), Temple orange, and certain other citrus varieties and citrus relatives in Florida and in Texas.

Evidence presented here indicates that control of Cachexia depends on selection of budwood free of the causal agent. Presence of cachexia in symptomless carriers may be determined by grafting them with cachexia-free scions of Orlando tangelo or other variety that readily expresses symptoms. Another method is to test suspected trees by inserting buds from them in seedling Orlando tangelos. Either of these methods requires several years to complete, but once established cachexia-free trees will serve as continuing sources of clean budwood.

SUMMARY

Cachexia has been transmitted many times under controlled conditions by means of buds taken from typically diseased trees, indicating that the causal agent is widely distributed throughout the framework of affected trees, even though the damage to the conducting tissues seems to be confined largely to the region of the bud union. At the same time and under the same cultural conditions trees propagated from healthy parents remained healthy. Seedling Orlando tangelo trees were infected by means of infected budwood; but, in close proximity, unbudded Orlando seedlings and those budded with healthy buds remained healthy, indicating no natural spread from tree to tree within the experiment.

Rootstock experiments in Florida and Texas showed that gum impregnation and discoloration of the phloem characteristic of cachexia may occur in mandarins, kumquats, and hybrids of either of these.

Evidence was found that at least 2 species of citrus (grapefruit and sweet orange) that do not express phloem symptoms may harbor cachexia, becoming, in effect, symptomless carriers of the causal agent of the disease. Presence of the causal agent of cachexia in symptomless varieties may be determined by inserting buds from suspected trees in seedlings of varieties that readily express symptoms, such as the Orlando tangelo. Establishment of known sources of healthy budwood appears to be an important means of control of this disease.

Comparison of the described symptoms, as well as examination of wood specimens of both diseases, indicates that cachexia and xyloporosis are the same disease. Comparison of host relationships of cachexia (xyloporosis) an tristeza adds to the evidence that these are distinct diseases.

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MULTIPLICATION OF WOUND-TUMOR VIRUS IN AN INSECT VECTOR [1]

L. M. Black and M. K. Brakke

Evidence for multiplication of certain plant viruses in their leafhopper vectors has been accumulated from experiments on passage of the virus through the egg for many generations (4, 3), on incubation periods following thermal inactivation of virus in infective leafhoppers (5, 6), and on titrations showing virus increase in insects during the incubation period (1). Recently, Maramorosch (8) has reported 10 serial passages of aster yellows virus, *Chlorogenus callistephi* Holmes through the vector, *Macrosteles divisus* (Uhl.) without replenishment of virus from plants. The present paper reports results of studies in which wound-tumor virus, *Aureogenus magnivena* Black was passed in series from vector to vector living on immune plants without decrease in virus concentration.

MATERIALS.—Experiments were conducted with the wound-tumor virus and one of its insect vectors, *Agallia constricta* (Van Duzee). Insects used in the serial passages were maintained on Grimm alfalfa, *Medicago sativa* L., which is immune to wound-tumor virus. Insects were tested for infectivity on crimson clover, *Trifolium incarnatum* L.

EXPERIMENTAL PROCEDURE.—Sixty vigorous adult insects that had fed on infected crimson clover for a month and on healthy crimson clover for the following three weeks were chosen as the original virus source for the experiment. The insects, having a total weight of 0.1270 gm., were placed in a cold room maintained at approximately 0° C. All succeeding operations up to the time when injected insects were returned to the greenhouse were performed in this room. The insects were thoroughly macerated by means of a glass mortar and pestle, with a small amount of saline (a solution containing 0.225 M. sodium chloride and 0.01 M. neutral potassium phosphate buffer) to aid maceration and prevent desiccation. Enough additional saline was added to give a final dilution of $10^{-1.5}$, e. g., a total of 3.9 ml. saline was added to the 0.1270 gm. of original source insects. The extract was clarified for 20 min. at 3400 rpm. in an International 1-SB centrifuge with swinging cups. The supernatant between the surface layer of fat and the precipitate was removed by means of suction through a capillary pipette and further clarified by an additional centrifugation. The second supernatant was used to make various dilutions in the buffered saline for the injections.

Pyrex glass tubing 3 to 4 mm. in diameter was used to make the capillary injection needles. The tubing was pulled out twice over a flame, the second time over a microburner to give fine points. The finer part of the capillary was kept shorter than 0.6 cm. since otherwise it was too flexible to permit exertion of pressure on the point at the desired angle. The points were broken by forceps under a Bausch and Lomb linen tester, No. 81-34-46 (fig. 1A), in such manner as to produce a sharp jagged end that entered the insects easily. After the points were broken, air was blown through the needles into a beaker of distilled water, the size of the bubbles giving a rough measure of the bore of the needle. Needle points were about 0.08 mm. in outside diameter. A needle already tested and found to be satisfactory was used as a guide at various stages in the process of manufacture.

A few minutes before beginning the injections, insects were placed in the cold room in order to immobilize them. About 15-30 leafhopper nymphs in the later instars were deposited in one part of the field under the lens of the linen tester placed on a green blotter. In order to prevent the insects from becoming active during the inoculations, it was necessary to use a low wattage fluorescent light source and to avoid breathing directly on the insects. The operator wore an army aviator's suit that kept him warm in the cold room for about an hour at a time. The insect to be injected was removed to the center of the field by means of the simple manipulator (Fig. 1B); the capillary needle (Fig. 1C) was inserted into the ventral side of its abdomen, and a small amount of the virus extract forced in by air pressure from the mouth. When the abdomen of the insect was observed to swell slightly at the injection, the insect, impaled on the needle, was moved to another part of the field and gently pushed off by means of the manipulator. The procedure was repeated until all 15-30 insects were injected without moving the injecting needle or the manipulator from the field of the lens. After all the insects were injected, they were moved to a test tube where they were allowed to warm to room temperature before being returned to a plant. A fresh needle, blotter, and manipulator were used with each different virus extract. Inoculations were made in order from the most to the least dilute virus solutions.

Measurements at each passage indicated that the average volume injected into each insect varied from about 0.05 μl. to 0.17 μl., the volume most frequently injected being 0.09 μl. When desirable, the total volumes of the solutions injected were measured in the following manner. A sample of the solution was drawn into a 0.1 ml. pipette calibrated in 0.01 ml. The pipette was held almost horizontal in a clamp, and the level of the meniscus adjusted by tapping the tip with filter paper. To fill an injection needle, the

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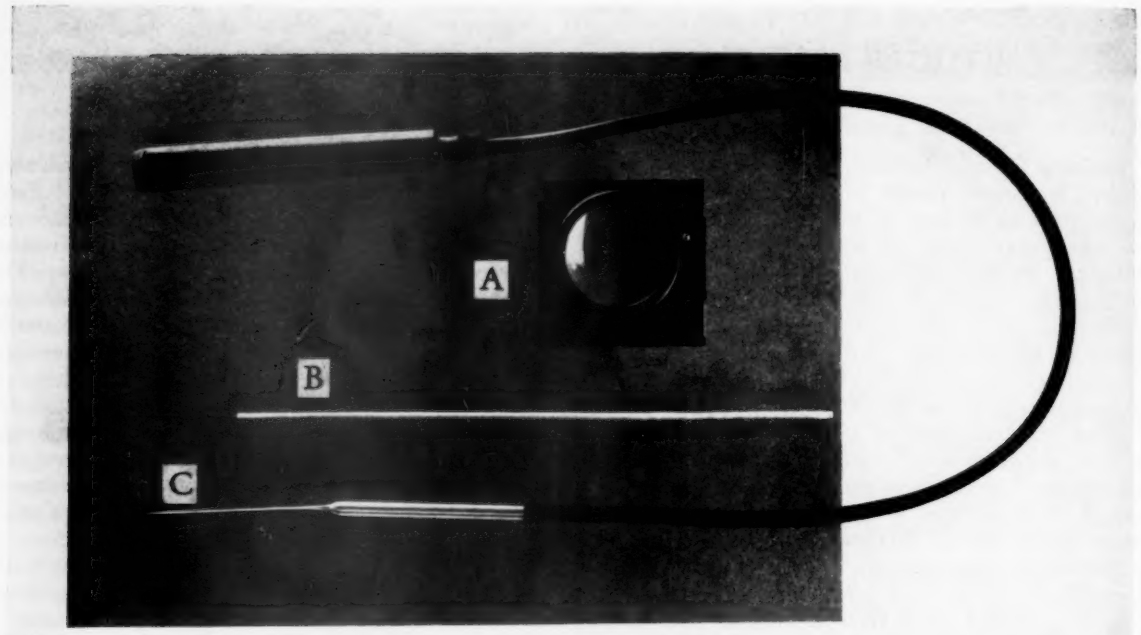

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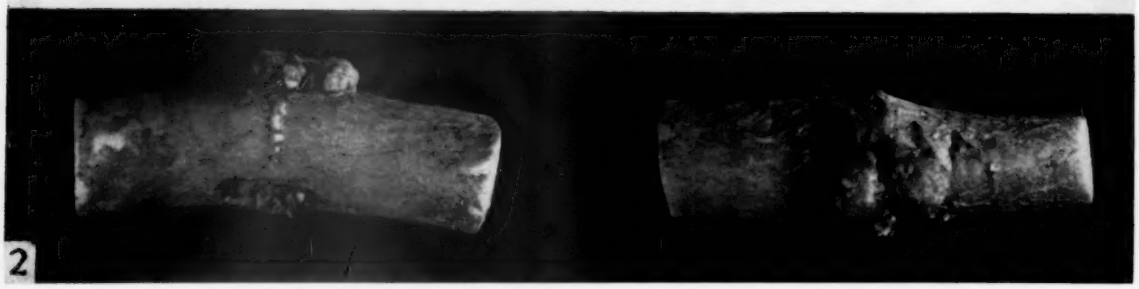

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FIG. 1. Apparatus used for injecting leafhoppers: A. Linen tester which was placed on a green blotter such as shown in the figure. B. Manipulator consisting of an insect pin inserted into a wooden applicator. C. Capillary glass needle with rubber tube and mouthpiece. (× 0.55) (Photograph by Louis Buhle)

FIG. 2. Sweet clover tumor growing from a small slice of infected tissue that had been inserted into an alfalfa stem 8½ months previously. The same tumor is shown in 2 views. The alfalfa did not become infected with wound-tumor virus from the scion tumor. (× 1.85) (Photograph by J. A. Carlile)

pipette was tilted until a small drop appeared on the tip and the needle was filled from this drop. When the pipette was levelled again, the drop ran back into the pipette and a mm. ruler was used to measure the distance the meniscus had moved. Because considerable fluid often leaks out after the injection, one can be sure that the total amounts retained in the insects were less than the amounts measured. At each passage, 30 insects were injected with each dilution except with the most concentrated solutions, that is, the $10^{-1.5}$ or 10^{-2} dilutions, which were injected into about 200 insects, some of which served as a source of virus for further passages and will be referred to as SPV (source of passage virus) lots. The 30 insects injected with a given dilution were placed on a caged Grimm alfalfa plant growing in a 4 in. pot of composted soil that had been steamed to kill all weed seeds. In each passage, the SPV insects were divided equally among 4 alfalfa plants. No weeds grew in any of the pots during the experiment.

The insects were allowed to feed for 2 wk. on the immune alfalfa. Then a sample of about 30 insects from the SPV lot and all the insects surviving the other injections were tested on crimson clover. Each insect was placed on a caged crimson clover seedling and allowed to feed on it for 1 month. The plants were then fumigated in a small room by means of a tetraethylpyrophosphate aerosol bomb and placed in a greenhouse for observation over a period of 6 wk. The greenhouse was fumigated at least once a week with tetraethylpyrophosphate in order to kill any nymphs hatching on the test plants before such insects had an opportunity to become infective. The remaining insects in the SPV lot were fed on alfalfa for an additional 2 wk. after which they were used as a source of virus for the next passage.

TABLE 1.—*Serial transmission of wound-tumor virus through an insect vector (Agallia constricta) grown on immune alfalfa*

Passage	Infectivity of insects inoculated with insect juice at indicated dilutions: 10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	$10^{-1.5}$	Fraction of original virus used as source for each dilution series (minus log).	Uninoculated Control Insects
1	0/24[a]	0/21	0/17	1/20	1/20	6/20	9/30	0.00	0/30[a]
2	–	–	0/28	1/28	5/27	–	6/28	3.06	0/27
3	–	–	1/16	3/20	4/20	–	4/10	6.05	0/20
4	–	–	–	0/20	3/27	11/27	–	8.76	0/24
5	–	–	–	1/27	5/29	11/30	–	12.13	0/25
6	–	–	–	0/25	8/28	15/28	–	15.24	0/27
7	–	–	–	2/30	8/28	17/30	–	18.32	0/28
Total	0	0	1	8	34	60	19		0
Infective	24	21	61	170	179	135	68		181
Percentage Infective	0	0	1.6	4.7	19.0	44.4	27.9		0

[a] Numerator equals number of insects infective; denominator, the number of insects tested.

During these experiments, the temperature at which the insects and plants were grown was maintained as close to 25° C. as possible by manual manipulation, since the work of Maramorosch (7) indicated that below this temperature the incubation periods in insects and plants increase markedly and the transmission of virus to plants by viruliferous insects becomes less frequent. The greenhouse was heated at night until summer night temperatures provided approximately the same conditions, and heating was renewed in the early autumn as soon as night temperatures fell below 25° C. for more than an hour or two. A 15- or 16-hr. day was maintained for the caged insects by means of banks of fluorescent lights and for the test plants in the greenhouse by means of 500-watt incandescent lights. Lights were also turned on during cloudy days.

SERIAL PASSAGE OF THE VIRUS IN THE INSECT VECTOR.—Results of the experiment are summarized in Table 1. Data on infectivity of insects of the first passage show that the original virus source was infective at dilutions $10^{-1.5}$, 10^{-2}, 10^{-3} and 10^{-4} but not 10^{-5}, 10^{-6} or 10^{-7}. Consequently, the last 3 dilutions were dropped from titrations in later passages. In this passage as in subsequent ones, noninoculated control insects, from the same source as the insects selected for injection, failed to infect any plants.

The fraction of original virus used as a source for each dilution series at the successive passages was calculated in the following manner. In the first passage 0.127 gm. of macerated viruliferous *Agallia constricta* leafhoppers (the original source) were diluted with 3.9 ml. of buffered saline to give a dilution of $10^{-1.5}$; 9.2 μl. of this solution was injected into 97 virus-free nymphs. After feeding on alfalfa for a month, 37 of the survivors were used as a source of virus to make up dilutions for the second passage. These 37 leafhoppers contained:

$$\frac{9.2}{(3.9 + 0.127) \times 10^3} \times \frac{37}{97}$$

or $1/10^{3.06}$ of the original virus. (Table 1).[3] Although the original virus had been diluted to approximately 10^{-18} in the 7th passage, there was no evidence of decrease in virus content of the insects. It should be remembered that the SPV insects had fed only on alfalfa during the experiment.

On the assumption that the virus content at each passage was approximately the same, the total number of infective insects for all passages at each dilution was obtained and expressed as a percentage of the insects inoculated (Table 1). With the exception of the $10^{-1.5}$ dilution, these percentages revealed that the method gave quantitative information, the percentage of infective insects being less at higher dilutions. Actually the logarithms of the percentages of infective insects fall virtually on a straight line when plotted against the logarithm of dilution, over the range 10^{-2} to 10^{-5}, indicating that the method is capable of giving quantitative assays.

IMMUNITY OF ALFALFA.—There is abundant evidence that Grimm alfalfa is immune to wound-tumor virus. In 3 different experiments, small slices of tissue from sweet clover tumors caused by the wound-tumor virus were inserted into the slit stems of 33 Grimm alfalfa plants and bound with Sterilastic. The alfalfa plants were pruned to the single stem bearing the graft. Only 4 of the tumor slices grew. One of these survived 6 months, when the alfalfa plant died from other causes. The other 3 grew very slowly over a period of 8½ months when the tops of the plants were cut

[3] In the second and third passages, one lot of about 100 insects was injected with a $10^{-1.5}$ solution passed through a Jenkins bacteriological filter with a 10 mm. filter block. This was done to eliminate a possible deleterious action from microbes and necessitated separate calculations of the dilutions attained for the filtered solutions in these passages. No harmful effect was noted from the unfiltered solutions and in the fourth passage, the filtered and unfiltered series were combined and the experiment continued without filtration. In doing so, the lower total dilution attained (the filtered series) was used as the basis for further calculations.

off and carefully examined. The best of the 3 scion tumors is shown in Fig. 2. None of the tops of the 3 plants showed any symptoms of wound-tumor disease. New growth from roots of these plants was allowed to develop for 10 months after which each plant was tested by feeding 60 virus-free nymphs on it for 3 wk. and testing them subsequently on each of 2 sets of 2 crimson clover plants for 2 wk. None of the 12 test plants developed wound-tumor. Corresponding control colonies fed on diseased crimson clover and tested similarly infected 8 out of 12 clover plants. Results indicated that Grimm alfalfa is immune to wound-tumor virus when grafted with sweet clover tumor tissue containing the virus.

Recently it has been demonstrated that when genetically marked (9) females of *Agalliopsis novella* (Say) carrying wound-tumor virus were allowed to lay their eggs in alfalfa plants, about 1 or 2 per cent of the progeny were infective (unpublished data). Virus-free normal females mixed with the viruliferous ones on the same plants gave rise only to virus-free progeny, although both the virus-free females and their progeny fed on the same alfalfa plants at the same time as their viruliferous counterparts. When viruliferous normal females were mixed with virus-free marked females, only the normal progeny contained viruliferous individuals. This type of experiment provides evidence that the immunity of alfalfa is such that virus cannot be passed from infective insects to virus-free insects feeding at random positions on the same plant and grown under conditions like those of the experiment on serial passage.

Twenty to 53 days after removal of the SPV insects, each of the 20 alfalfa plants used to support them in passages 3 through 7 was tested for virus. These plants had not been pruned between the removal of the SPV insects and the beginning of the test. About 50 small virus-free nymphs were fed on each plant for a month. Approximately 45 surviving insects from each plant were divided into 5 colonies, each of which was tested on a crimson clover plant for 2 wk. The clover was subsequently observed in the greenhouse for 6 wk. As controls, infected crimson clover plants were tested in exactly the same manner. Only 1, plant X, out of 100 test plants was infected by insects from alfalfa while 92 out of 100 were infected by insects from diseased clover. The infection on plant X is believed to have been due to contamination and not to virus from the alfalfa because other contaminations occurred in the greenhouse at this time. Another indication of contamination was the fact that plant X developed symptoms at the end of the 6 wk. observation period in the greenhouse whereas all but 1 of the control plants had developed symptoms after only 17 days.

These same 20 alfalfa plants were subsequently retested. To each of 10 of the plants were added 40 nymphs of *A. constricta*, fed for the previous 17 days on crimson clover infected with wound-tumor. Under the conditions of the experiment wound-tumor virus has an incubation period of 2 weeks in its vectors (7). To each alfalfa plant were also added 40 virus-free nymphs of *Agalliopsis novella*, another vector of the virus (2). After feeding together on the alfalfa plants for 19 days, the insects were removed and the 2 species separated. Twenty insects of each species from each alfalfa plant were tested for the virus by feeding in colonies of 10 on crimson clover plants for 16 days. These plants were then observed in the greenhouse over a period of 6 wk. The remaining 10 alfalfa plants were tested in the same manner except that the viruliferous insects were *A. novella* and the non-viruliferous, *A. constricta*. Of 40 clover test plants on which the viruliferous insects fed, 39 were infected; of the other 40 on which the virus-free insects fed, 1, plant Y, became infected. For a number of reasons plant Y is believed to have been infected by viruliferous leafhoppers contaminating the greenhouse. The colony that had fed on plant Y came from a different alfalfa plant than did the colony that fed on plant X.

The contaminations in the last 2 experiments are believed to have resulted from a minor change in technique. Sometime previously viruliferous vectors were found in the greenhouse feeding on large diseased clover plants. These plants had been inoculated at a later stage of development than in earlier procedures in an effort to get a better source of virus. However, the canopy of foliage formed by the larger plants growing close together had protected the insects from the fumigant used regularly in the greenhouse.

It is concluded from these tests that Grimm alfalfa is immune to wound-tumor virus.

DISCUSSION AND SUMMARY

During 7 serial passages of wound-tumor virus through *Agallia constricta*, the virus in the insects was not replenished from the immune alfalfa plants on which the leafhoppers were grown. The dilution of the quantity of virus with which the serial passages was started exceeded 10^{-18} when the series was finished, yet there was as much virus present at the end as at the beginning. This conclusion is strengthened by the evidence that the amount of virus was roughly the same at each of the 6 preceding passages. It is concluded that the virus must have multiplied in its insect vector. These results parallel those reported recently for aster yellows virus by Maramorosch (8).

The logarithm of the percentage of leafhoppers rendered infective by different virus suspensions was in direct proportion to the logarithm of the virus concentration.

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STUDIES ON STREPTOMYCES SCABIES. I. VARIABILITY IN A MELANIN-INDICATOR MEDIUM [1]

John P. Hollis

It has been previously reported (8) that yeast autolysates and vitamin-free casein hydrolysates stimulate melanic pigment production by *S. scabies* in a tyrosine medium. This communication reports the stimulatory effect of other enrichment substances and records the segregational and mutational characteristics of a strain of the organism in a melanin-indicator medium.

MATERIALS AND METHODS. — *Cultural methods.* — *Streptomyces scabies* (Thaxt.) Waks. and Henr. strain Hooker No. 5 (9) and its derivatives were maintained on potato dextrose agar (PDA) slants by mass transfer at 3 wk. intervals. Spore suspensions were prepared from these slants, aged 2 to 3 wk., by suspending surface growth in 10 ml. sterile water and filtering through absorbent cotton to remove mycelial and agar fragments. Counting chamber determinations indicated spore yields in excess of 40,000,000 per ml. in these filtered suspensions. Dilutions were made to yield 100 to 200 colonies per plate when approximately 0.05 ml. was spread over the agar surface with a glass rod. Colony types were easily differentiated after incubation periods ranging from 5 to 10 days.

Melanin-indicator medium.—The minimal melanin-indicator medium is a modification of Afanasiev's medium (1) and contains in g/1: KNO_3 1.52, $MgSO_4 + 7H_2O$ 0.49, K_2HPO_4 1.20, $CaCO_3$ 0.02, tyrosine 1.00, glucose (anhyd.) 8.00, agar 15.00, H_2O 1000 ml. and metals solution[2] 1.00 ml. to yield final concentrations of each metal of 0.01 γ/ml. The complete melanin-indicator medium contained a supplement, usually Difco yeast extract or one of the following substances: Difco peptone, Difco tryptone, Sheffield NZ-case, NBC[3] "vitamin-free" acid or enzymatic casein hydrolysate. Solutions of enrichment substances were sterilized in the autoclave and 1 ml. quantities were added aseptically to the melted minimal medium in the plates.

EXPERIMENTAL RESULTS. — *Stimulation of melanogenesis.*—Pronounced growth stimulation was evident after 3 days incubation of plates supplemented with yeast extract, peptone, NZ-case and tryptone in 1.0 per cent final concentration whereas growth was barely noticeable in plates supplemented with enzymatic casein hydrolysate and was undetectable in those with acid casein hydrolysate. These sharp differences disappeared after 5 to 8 days incubation and both the latter substances were stimulating at final concentrations of 0.25 and 0.5 per cent but inhibitory at 2.5 per cent. Pigment formation was strongly correlated with growth. The use of ascorbic acid in these experiments is justified in that its addition to tissue oxidase systems is known to result in its preferential oxidation with consequent protection of the usual substrate, in this case tyrosine. Examination of the ascorbic acid-effect shows a reduction in both melanogenesis and growth with increasing concentration. Although melanin formation is generally correlated with age and a loss of reducing ability in tissues of higher plants, and increase of cellular oxidase activity consequent to the loss of structural integrity and dehydrogenase activity in bacteria (13) opposite relations are evident in the present experiments.

Addition of yeast extract and acid casein hydrolysate resulted in a melanin diffusion pattern (Fig. 1) characterized by intense coloration immediately surrounding the colony. Peptone, tryptone, NZ-case and enzymatic casein hydrolysate, on the other hand, stimulated diffusion of melanin from the colonies throughout the medium and were therefore not so useful for differentiating melanogenic types. Yeast extract in 0.1 and 1 per cent final concentrations was used most frequently for colony differentiation because of its superior growth stimulating properties.

Colony types on melanin-indicator medium.—Three basic types of colonies developed when the parent culture was assayed on the minimal medium supplemented with yeast extract. Figure 1A shows the differentiation achieved with yeast extract at 0.1 per cent final concentration. Type A colonies are small with a narrow and intense melanin-diffusion ring. Aerial mycelium is usually absent at 3 days but may develop abundantly after 5 days. Type B colonies are large with weak melanin-diffusion zones and scant to abundant aerial hyphae developing in 3 to 5 days. Type C or albino colonies are gray or white with variable aerial mycelium, and are further characterized by a lack of exogenous melanin production, although vari-

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[2] 10 γ/ml. each of Mn, Fe, Zn, Cu, Co, Mb, and Ga in a salts mixture.

[3] Nutritional Biochemicals Corporation.

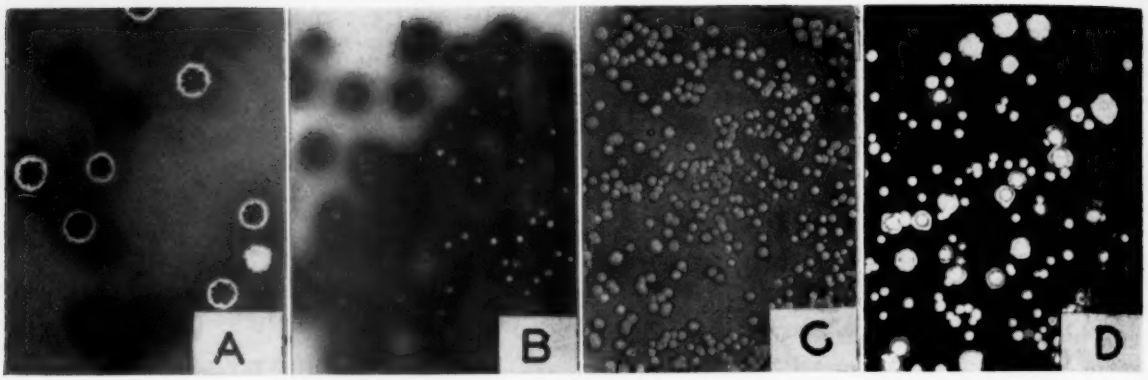

FIG. 1. Colony types of *S. scabies* on melanin-indicator media. A—Colony types A, B and C segregation from parent culture. B and C—Pure cultures of colony types A and B respectively grown from monocolonial selections derived from daughter cultures originally selected from the parent. D—Culture of a type C monocolonial selection from the parent isolate.

ants may exhibit some darkening of spores. Melanogenesis by types A and B is somewhat stronger on media supplemented with 1 per cent final concentrations of the stimulating substances (Fig. 1B, C).

Equivalence of the supplements relative to colony types.—When the parent culture was maintained on PDA and transferred at 2 to 3 wk. intervals, plating on the melanin-indicator medium containing yeast extract yielded albino colonies at a relatively constant rate. Experiments were made to determine whether various suplements would exert differential effects on the rate of appearance of albino colonies. Ten ml. of minimal medium was supplemented with (a) peptone, NZ-case and yeast extract, all at 0.1 per cent final concentrations and (b) acid casein hydrolysate at 0.05 per cent final concentration. In Table 1 are data from three successive experiments. All suspected albino colonies were transferred to duplicate melanin-indicator slants containing supplements of yeast extract and NZ-case for verification. Absence of major differences in per cent of albino colonies and consequently in per cent of A and B types combined suggests a stimulatory principle common to all enrichment materials tested.

Accumulation of albino colonies on melanin-indicator medium.—Seven months of culturing *S. scabies* on the 0.1 per cent yeast extract melanin-indicator medium with periodic transfer resulted in certain cultural changes of interest. Aerial hyphae decreased and sporulation dropped off markedly. Erikson (3) reported cultures of actinomycetes in synthetic media lost capacity to produce aerial mycelium and spores as compared with cultures kept in soil. In the present work, upon transferring *S. scabies* back to PDA for carriage by successive serial transfer, there was a gradual decrease in the proportions of albino colonies over a 120 day period (Fig. 2), leveling off at 0.5 to 0.6 per cent from a high of 8.5 per cent. The lower levels of occurrence approximate those for mutants from monocolonial derivations of the parent strain and, therefore, the higher levels of albino colonies must represent

TABLE 1. *Per cent albino (type C) colonies of S. scabies segregating from the parent culture on melanin-indicator medium composed of various enrichment substances*

Experiment	Supplement	Supplement conc. (per cent)	Total colonies	Per cent albino colonies
1	peptone	1.0	760	0.13
	yeast extract	0.1	1090	0.18
2	acid casein hydrolysate	0.25	2586	0.80
3	peptone	1.0	164	1.16
	NZ-case	1.0	514	0.97
	yeast extract	0.1	269	0.00
	acid casein hydrolysate	0.25	1838	0.60

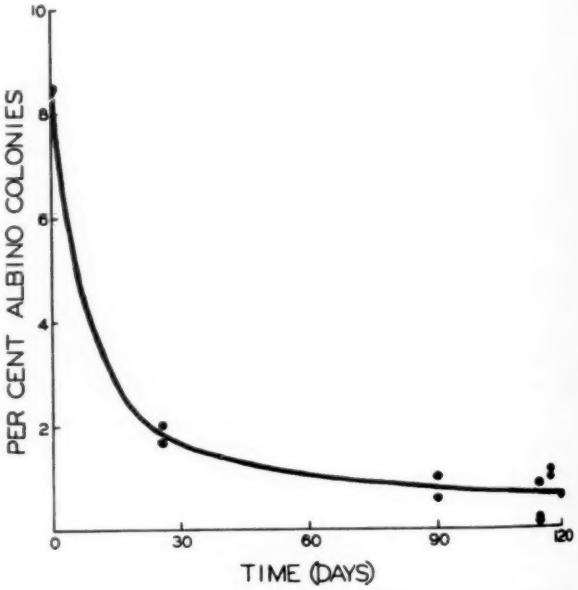

FIGURE 2. Reversal of the accumulation of the albino type of *S. scabies* on potato dextrose agar after 7 months previous culture on a melanin-indicator medium.

a selective accumulation of mutants, produced during the period of culture upon the synthetic medium.

Mutants from monocolonial isolates.—The foregoing data do not reveal the mechanism of the variation giving rise to the 3 colony types. Furthermore, nothing is known of the original constitution of the parent cultures with regard to melanin production. Accordingly, several successive experiments were conducted: the first involved plating of A, B and C monocolonial selections from the parent culture; then in turn, the plating of similar monocolonial selections from the daughter and granddaughter cultures. Results (Table 2) reveal a persistent reappearance of albino, type C colonies from all selections of the melanogenic types A and B, explicable only on the basis of mutation. Figure 1B, C, D shows the constancy of types A and B selected from daughter cultures and type C (albino) from the parent strain. The range of variability of A and B colony types derived from the parent culture (not shown) was somewhat greater than the selections from daughter colonies shown here. Such variability is probably related to the longer period of culture of derivatives of the parent strain and the concomitant accumulation of mutants through selection. Studies of mutation from A to B and vice versa have not been made.

TABLE 2. *Per cent albino, type C colonies occurring in plants inoculated with melanogenic derivatives A and B of parent, daughter and grand-daughter selections.*

Colony type	Culture	Source	Total colonies	Per cent albinos
A	108	Parent	1033	0.29
A	109	Parent	1211	0.41
B	112	Parent	1893	0.58
B	113	Parent	2449	0.21
A	221	108	1984	0.20
B	223	112	3429	0.41
A	249	221	2244	0.36
B	251	223	385	1.04

DISCUSSION.—Afanasiev (1) showed melanogenesis by *S. scabies* to be conditioned by cultural factors of pH and balance between the concentrations of tryosine and certain inorganic nitrogen compounds—KNO_3, $NaNO_3$ and $(NH_4)_2CO_3$. The present work, extending a previous report (8), indicates the stimulating principle to be common to a variety of enrichment substances.

Although mechanisms of both inorganic and organic stimulation of melanin formation remain to be elucidated, certain facts are already apparent. The effective concentration of tyrosine (0.1 per cent) constitutes a stable supersaturated solution[4] at ordinary temperatures. Concentrations at saturation (0.04 per cent) or below, are limiting to melanogenesis in *S. scabies*. In addition to the sources of variability elucidated above, mutation to form colony types A, B and C and their segregation from the heterokaryon, there remains the phenomenon of physiological degeneration and formation of cultural mutants[5]. These were evident as cultures carried on PDA which had lost ability to produce melanin in minimal medium while the same isolates carried in soil cultures produced abundant melanin in the absence of organic supplements.

Such facts suggest that the production of melanin is but another example of overflow on shunt metabolism (5), a concept which aids in formulating probable mechanisms of action of the organic stimulatory effect in melanogenesis. The melanogenic reaction is closely linked with tyrosine concentration and it is probable that the stimulative principle does not function as an intermediate metabolite in the reaction chain. Although phenylalanine as a normal precursor of tyrosine was ineffective (8), the possibility remains that tyrosine concentration may be maintained at abnormally high levels during the reaction by its simultaneous productions from peptides or other amino acids with which it is in chemical equilibrium. A more likely possibility is that the factor or factors responsible for stimulation may function in the synthesis of tyrosinase, as either a constituent of the enzyme or indirectly in other syntheses. The stimulatory substances may complete with tyrosine and its precursors for surfaces of the enzymes involved in synthetic reactions and by inhibiting the normal synthetic metabolism of these substances, encourage the alternative shunt reaction to melanin formation. On this basis the inhibitory effect of ascorbic acid on melanogenesis and growth results from the "protection" of tyrosine from oxidation and the consequent antagonism of tyrosine in high concentration for other amino acids in competition for the active surfaces of enzymes catalyzing protein syntheses.

Problems of differential solubility, in separating natural melanin extracted from complex tissues from contaminants, have retarded advances in knowledge of melanin chemistry. The present reaction yields a large supply of water-soluble pigment, for chemical determinations, which is relatively free of contaminants. Proof that the pigment is melanic in nature rests upon the following facts: excretion of pigment is quantitatively linked with tyrosine dosage and behaves toward pH in a fashion characteristic of the tyrosinase reaction *in vitro*. Further the pigment is precipitable by $FeCl_3$ in the presence of phosphates, a test reported by Hawk, Oser and Summerson (7) as satisfactory for identification of melanin in urine.

Experimental materials used in this work are the most efficacious reported to date for study of the factors influencing melanin production *in vivo* (6).

The mutability of *S. scabies* in melanogenic power

[4] Addition of a trace of tyrosine to this solution results in the formation of an abundant precipitate of tyrosine crystals.

[5] Cultures carried on ordinary laboratory media (PDA) which exhibit physiological or other properties different from fresh isolates or from clonal comparatives carried in certain media such as soil.

is not surprising since numerous workers have demonstrated spontaneous occurrence of variants in several species of *Streptomyces* with regard to both morphologic and physiologic characteristics, (10), (15), (16), (17), (18). Jones (11) studied variation in pigment production of single cell cultures of certain saprophytic soil actinomycetes. Although dark pigments were produced by the isolates, the media used to culture the organisms could give rise to other types of pigment than melanin. Both pigmented and colorless colonies were obtained from single spore selections but the nature of the variation was not elucidated.

If one assumes polygenic control of the tyrosinase reaction (12), then the numerous albino mutants obtained in the present work furnish tools for attempts at recombination of factors responsible for melanogenesis in this organism. Other strains will undoubtedly be shown to be mutable for the albino condition. In a test for nonallelism involving 380 possible combinations among 20 albino isolates derived from the parent culture, results were negative. Absence of hyphal fusions generally among actinomycetes was noted by Erikson (3), a fact which indicates that, in an experiment of this type, only positive results have meaning. Negative results may have been due to the fact that all the albino isolates were allelic, nonfusing or double mutants.

SUMMARY

Streptomyces scabies forms abundant melanin pigment in a tyrosine medium supplemented with 0.1 to 1 per cent concentrations of any of the following substances: yeast extract, peptone, tryptone, NZ-case, acid casein hydrolysate, enzymatic casein hydrolysate. Three types of colonies were derived from the parent isolate on melanin-indicator media. Type A is strongly melanogenic, whereas type B is intermediate with respect to type A, and the albino type C colonies in production of melanin. The albino type arises by mutation from both types A and B at levels averaging 0.2 to 1 per cent of total colonies assayed.

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LATE BLIGHT IMMUNITY IN THE POTATO [1]

F. J. Stevenson, E. S. Schultz, R. V. Akeley, and L. C. Cash [2]

In breeding potatoes for immunity from late blight, caused by *Phytophthora infestans* (Mont.) DBy., tens of thousands of seedling varieties and many named varieties of *colanum tuberosum*, both American and foreign, have been tested. Various degrees of resistance have been found, but it is evident that all varieties of this species are susceptible in some degree to late blight; in many, however, the resistance is high enough to be of commercial importance. At an early date some wild species of *Solanum* showed an immune reaction, and a number were reported to be highly resistant to if not immune from the disease (10).

Salaman (8), working with *Solanum edinense* in 1906, discovered genuine resistance to late blight. From 1906 to 1910 this species proved almost but not quite immune from attacks of blight. *S. edinense* plants were self-fertilized, and 40 plants grown from the resulting seed were tested in 1909 for their blight reaction. Blight was bad that year and again in 1910 so that all cultivated varieties or new seedlings derived from them were killed. Thirty-three of the *S. edinense* seedlings were also killed, but 7 remained untouched. These continued to show immunity in the years that followed. One of them was grown in the garden at Barley, England, for 17 consecutive seasons and never showed the least sign of infection with blight. Satisfied that true resistance could be found only outside the domestic varieties, Salaman succeeded in obtaining several other wild species including *S. demissum* from Mexico. In 1910 a selfed line of *S. demissum* tested for blight showed an immune reaction. Hybrids were made between *S. demissum* and *S. tuberosum*. By 1914 a series of such hybrid families were under test, and some of the most resistant of these were crossed with immune sorts derived from *S. edinense*. By 1926 more than 20 seedling varieties with reasonably good economic characters combined with blight immunity were available. A few of these were sent to Ireland where they have been grown in fields for more than 20 years and have retained their high degree of resistance.

According to Salaman (8), similar work was begun in 1914 by Broili at Dahlem, Germany, and was followed up in 1920 by K. O. Müller. Müller discovered immunity in a group of seedling varieties derived from species hybrids. These were designated the *WS* races, or, as we now call them, the *W* varieties. The wild progenitor of these varieties is not definitely known, but is thought to be *Solanum demissum*, since the reactions of the *W* varieties and of the American variety Kennebec, which inherited its resistance from the *W* varieties, show that they carry a gene for immunity identical with one of the factors carried in *S. demissum*. The original parent was obtained by Professor Wittmack, of Berlin, from the United States Department of Agriculture in Washington. According to Reddick (7), when Professor Wittmack turned over his samples to workers at Dahlem, one with a mutilated label was marked "WS." This is an abbreviation for Washington Sammlung, and it was this sample that proved highly resistant to blight. *S. demissum* has never been found outside Mexico (6). It was brought to Vermont by Dr. Pringle and was grown there among several other wild species by William Stuart before he took charge of the potato-improvement program in the United States Department of Agriculture. Some of the species in this collection may have been sent to Germany.

In the United States the work of breeding blight-resistant potato varieties began about the middle of the nineteenth century. While many excellent varieties, some of which are today being grown commercially, were produced during the last half of the century, none was resistant to late blight. Protective measures that permitted the growing of susceptible varieties were devised, and the work of breeding for blight resistance was allowed to lapse for a time. In 1910 potato breeding was actively undertaken by the United States Department of Agriculture under the leadership of Dr. William Stuart. At that time, the only disease resistance sought was to the late blight fungus (2). However, the work had not progressed very far when it became evident that virus diseases had to be given chief consideration, and it was not until 1932, after the present national potato-breeding program was under way, that emphasis could be placed once more on breeding for resistance to late blight. Dr. Donald Reddick of Cornell University had already undertaken the solution of this problem by using species crosses with special emphasis on *Solanum demissum* hybrids.

Since 1932 the work of breeding potatoes for resistance to or immunity from late blight, which is conducted by the United States Department of Agriculture in cooperation with the Maine Agricultural Experiment Station, Presque Isle, Maine, has gone through 4 phases: The testing of varieties and seedlings of *Solanum tuberosum* to determine their reactions to late blight; the use as parents of foreign varieties showing an intermediate degree of resistance; the introduction of seed of the German W varieties, possessing the gene for immunity from the common physiologic races, and the use of these as parents in production of superior commercial varieties immune from the common races; and the use as parents of seedling varieties related to *S. demissum* but carrying 2 or more of its genes for immunity in an attempt to breed commercial varieties immune from all the physiologic races known at present.

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Results of importance have been accomplished in each of these phases. An intermediate degree of resistance was found in the selfed progeny of a susceptible variety and in a cross between 2 susceptibles. A selection from a cross of the 2 susceptible varieties, Chippewa and Katahdin, was found to be more resistant than either parent. Resistance was evident in both vines and tubers of this selection, and as its horticultural characters were satisfactory it was named Sebago and released to growers (9).

In the second phase of the work foreign varieties, such as President and Ackersegen, which show an intermediate type of resistance to late blight, were crossed with American varieties and seedlings. Many seedling varieties with about the same degree of resistance as the foreign parents were found. From this breeding, 2 varieties were released—Saranac (11), a selection from a cross of President × Katahdin, and Calrose (3), from a cross of Ackersegen × Katahdin. These both showed a high enough intermediate type of resistance to late blight to be of commercial value.

The third phase in breeding for blight resistance has also produced positive results. In 1934 we received from Dr. Müller seed of his *W* varieties. At the time he sent this seed he indicated that the material carried genes for immunity from the common forms of late blight. Seedlings were grown and tested for blight reaction. Many of the seedlings, both early and late, came through the tests at Aroostook Farm, Presque Isle, Maine, free from blight, whereas other seedlings and the susceptible checks were dead as long as 30 days before they would have matured normally. The tubers of the seedlings were small and, as a rule, rough; so from the standpoint of horticultural characters they all should have been discarded. However, a few of the best were saved and crossed with parents that were known to contribute desirable shape, shallow eyes, and other characters of economic value. Several early selections that showed the same reaction to late blight as the *W* varieties were made from one of those crosses and, from the standpoint of horticultural characters, were a big improvement over the *W* varieties. However, because they could not compete with standard varieties in yield another series of crosses was made, this time with high-yielding varieties, some of which had the intermediate resistance developed from crossing the 2 susceptibles in the first phase of this project. Again, a relatively large number of the seedlings showed an immune reaction in blight tests. Several of the best of these were selected and tested for yield and cooking quality and distributed to cooperating State Experiment Stations for adaptation trials. Three of these, Kennebec, Pungo, and Cherokee, have been named and released to growers. According to Dr. W. R. Mills of Pennsylvania State College, Kennebec reacts to his collection of physiologic races in a manner similar to some of his own and Dr. Reddick's varieties that have one of the genes for immunity inherited from *Solanum demissum*. This suggests strongly that the original wild progenitor of the *W* varieties was *S. demissum*.

With the release of Kennebec, Pungo, Cherokee, and a number of Dr. Reddick's varieties, such as Essex, Ashworth, and Placid, the blight problem in the United States would have been solved if it had not been for appearance of new physiologic races. About 20 years ago it was thought that there was only one biotype of the late blight organism in this country. In 1933, after testing *Phytophthora infestans* from widely different sources, Reddick and Crosier (5) concluded that either this organism as it occurred in most parts of North America was not biologically specialized or the varieties of potatoes that were being grown then did not reveal the presence of more than one race. It was not until varieties and seedlings related to *Solanum demissum* were produced and showed various reactions to the disease that different races of the late blight organism could be identified. Varieties such as Kennebec and Essex were immune from the common races of the late blight fungus but susceptible to the more virulent ones. Some of these races were found on the senile leaves of blight-resistant plants in field tests; others were produced under controlled conditions.

Virulent races used in testing for immunity at Beltsville, Md., were found on resistant varieties in the test plots at Presque Isle, Maine. The races used at Cornell University, Ithaca, N. Y., and State College, Pa., were produced under controlled conditions. With regard to the latter, Reddick and Mills (6) reported that they had induced an increase in virulence of the blight organism by passage of cultures successively through varieties each possessing a higher degree of resistance than the preceding one until a point was reached where a number of potato hybrids that previously had given immune reaction could be severely infected. How many races exist at present or how many will be found in future work is not known. Fortunately, *Solanum demissum* is immune from all the races known at present. The immunity of *S. demissum* is not inherited as a unit as was once supposed, but is controlled by multiple factors. This fact has been determined not from genetic ratios found in segregating populations but by reactions of seedlings related to *S. demissum* to different physiologic races of *Phytophthora infestans*.

Mills (4) stated: "A total of 3 distinct genes for resistance has been identified among the descendants of *Solanum demissum*. Intercrossing of plants carrying different genes will produce immunity to all of the races of the fungus which have been isolated from the field."

Black (1) employed 3 strains of the late blight organism, A, B, and C, in testing seedlings bred from blight-immune *Solanum demissum*. He divided the seedling varieties into 5 phenotypes according to their reactions to the 3 physiologic races: immune from A, B, and C; immune from A and B but susceptible to C; immune from A and C but susceptible to B; immune from A but susceptible to B and C; and susceptible to A, B, and C. Black wrote: "Insofar as the

A, B, and C strains are concerned blight resistance is a heritable character behaving in a dominant fashion, and it appears to be controlled by major genes as follows: Ra confers immunity from strain A; Rb confers immunity from strains A and B; Rc confers immunity from strains A and C; Rbc confers immunity from strains A, B, and C." In addition to the 4 main factors several minor genes were present. These minor genes modified the reactions of susceptible and resistant varieties.

Multiple genes for immunity found in *Solanum demissum* segregate or separate in crosses and backcrosses with cultivated sorts. Some of the resulting seedling varieties carry only 1 of the genes; others possess 2 or 3. These seedling varieties are far superior to the wild parent in horticultural characters. By the use of these improved sorts as parents we are attempting to recombine the several immunity genes and to produce commercial varieties that are immune from all the physiologic races of the late blight fungus now known. This is the objective of the fourth phase of the project. One of the first crosses we made in this latest phase of our work was between TI 5 and B 355-24. TI 5, one of Dr. Reddick's older seedlings, is 5 generations removed from *S. demissum*. It is immune from the common races of the late blight organism and from at least 3 of the more virulent races. It is fair from the horticultural standpoint but could not compete commercially with such varieties as Green Mountain or Kennebec. B 355-24 is immune from the common races of the late blight fungus and resistant to the ring rot bacterium. From the cross of TI 5 and B 355-24, 337 seedlings were grown. Of these 23 were selected as having commercial possibilities and tested for their reaction to late blight in the field. The vines of 11 of these showed an immune reaction, and their tubers when inoculated with blight spores failed to develop blight rot. Five of these escaped infection when inoculated with spores of 6 virulent cultures at Beltsville, Md. President, which shows an intermediate type of resistance to the common races, was severely injured by all 6 cultures and Kennebec, which seems to be immune from the common races, was attacked by 4 of the 6 races used in the test.

The 5 selections that showed immunity in the tests at Beltsville were sent to Cornell University where L. C. Peterson tested them with his races, B, C, D, BC, and BD. Three selections showed an immune reaction to B, C, D, and BD but not to BC. This is the same reaction shown by the parent TI 5. The other 2 were free from infection with all 5 races. One of the seedlings that escaped infection from both the Beltsville and Cornell cultures is promising commercially. It is being tested for yield, cooking quality, and other characters of importance.

Many other seedlings are being bred for immunity from the virulent races both here and in foreign countries, and in the not too distant future commercial varieties immune from all the races of the late blight organism that are known should be available.

DISCUSSION AND SUMMARY

In the United States and in foreign countries varieties of potatoes immune from late blight have been sought for more than 100 years. The problem of breeding such varieties is complex. If it were simple it might have been solved many years ago. A list of the factors contributing to the intricacy of the problem follows:

(a) There seems to be no immunity in the cultivated varieties, and most of them are highly susceptible.
(b) Immunity from all the known physiologic races of the causal organism is found in wild species possessing no horticultural characters of value.
(c) Most of the immune wild species are not crossable with cultivated varieties.
(d) Immunity from all physiologic races is dependent on multiple genes.
(e) Environmental factors cause variability in varietal reactions.
(f) There are a number of distinct physiologic races of *Phytophthora infestans*, which seems to be continuously mutating if given the proper environment.

Progress has been made in spite of the many complications. This is evident in our greater knowledge of the inheritance of blight immunity and in the information we have on physiologic specialization in the organism.

We have released to growers a number of varieties with an intermediate degree of resistance to late blight and others apparently immune from the common physiologic races. The varieties immune from the common races have maintained their resistance under a wide range of environmental conditions in most of the potato-growing sections of the United States. Occasionally, plants have become infected with what in some cases proved to be more virulent races than the common ones, but the infection was usually very light and occurred after the tubers were almost if not quite fully grown, so that there was little or no reduction in yield. Even if we fail to breed varieties immune from all races, a resistant variety on which blight does not appear until late in the growing season is of great economic value.

Several species of *Solanum* are immune from late blight but most is known about the immunity of *S. demissum*, because it is easily crossed and backcrossed to varieties of *S. tuberosum*. Some of the seedling varieties are immune from one or more of the virulent races after 5 backcrosses to cultivated sorts. Such varieties have been produced in the United States; others have been imported from Canada, Germany, and Scotland. Recently, 4 seedling varieties that are immune from all 3 of his late blight strains A, B, and C were received from Dr. William Black of Scotland. With all the material available, the fund of knowledge we now have, and the progress that has already been made it seems certain that in the near future there

should be produced varieties possessing the multiple genes for immunity found in *S. demissum* combined with the best characters of the commercial varieties.

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A PHYTOPATHOLOGICAL NOTE

Bud-take of healthy and tristeza-infected citrus buds. A. S. Costa, T. J. Grant, and Silvio Moreira. In the cooperative tristeza project[1] carried out at Campinas, Brazil, several tests were made to compare the growth of sprouts from healthy and tristeza-infected buds on different citrus rootstocks. Both types of buds usually took very well. However, in some experiments in which budding was performed under unfavorable conditions, buds from plants of certain citrus varieties showing early stages of decline, gave a better take than healthy ones. Data on bud-take[2] from these and similar tests, are presented here.

Virus-carrying buds of 4 varieties of sweet orange (*Citrus sinensis* (L) Osb.) were obtained from plants grafted on rootstock of sour orange (*C. aurantium* L.) and showing decline symptoms in the greenhouse. Virus-free buds were taken from comparable healthy plants of the same combinations. Infected and healthy buds of 2 other sweet-orange varieties were taken from field plants budded on sour-orange rootstock. Buds of Eureka lemon (*C. limon* (L.) Burm. f.), Columbia sweet lemon, and Dancy tangerine (*C. reticulata* Blanco) were obtained from healthy and diseased plants budded on sour-orange rootstocks in the field. Healthy and virus-carrying buds of West Indian limes (*C. aurantifolia* (Christm.) Swingle) and grapefruit varieties (*C. paradisi Macfad.*) were taken from unbudded greenhouse seedlings. The infected seedlings of these varieties showed definite decline symptoms. The buds taken from all these trees were inserted into rootstocks of 7 types. Most of the budding tests were carried out with field plants in the nursery, but a few tests were made with potted plants in the greenhouse.

Results of the tests (Table 1) indicate that tristeza-infected buds of various citrus varieties taken from

[1] Cooperative project between the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, and the Instituto Agronomico, Campinas, Brazil.

[2] Bud-take data are limited to growth of all buds that survived.

Table 1.—*Results of budding tests with healthy and tristeza-infected buds of various citrus types*

Scion	Bud condition	Number of buds tested/bud-take, on the indicated rootstock								
		Sweet orange	Sour orange	Rangpur lime	Cleopatra mandarin	"Lima da Persia"	"Cravo" tangerine	Rough lemon	Total	% take
Sweet orange (6 varieties)	Diseased	50/48	20/17	30/30	30/20	30/26	30/21	30/27	220/189	85.9
	Healthy	40/29	10/6	30/22	30/18	30/22	30/11	30/7	200/115	57.5
Grapefruit (6 varieties)	Diseased	45/38	30/29	15/14	15/11	15/14	15/6	15/3	150/115	76.7
	Healthy	30/29	15/15	15/12	15/8	15/15	15/7	15/5	120/91	75.8
West Indian lime (2 varieties)	Diseased	35/32	10/10	25/23	25/23	5/4	5/3	5/1	110/96	87.3
	Healthy	20/5	5/4	15/4	15/1	5/2	5/0	5/1	70/17	24.3
Eureka lemon	Diseased	5/3	–/–	5/4	5/3	5/3	5/1	5/0	30/14	46.7
	Healthy	5/0	–/–	5/5	5/0	5/4	5/0	5/0	30/9	30.0
Columbia sweet lemon	Diseased	5/3	–/–	5/3	5/1	5/2	5/1	5/2	30/12	40.0
	Healthy	5/0	–/–	5/0	5/0	5/1	5/0	5/0	30/1	3.3
Dancy tangerine	Diseased	5/1	–/–	5/2	5/0	5/5	5/0	5/2	30/10	33.3
	Healthy	5/0	–/–	5/0	5/0	5/2	5/0	5/0	30/2	6.7

plants showing decline symptoms gave a better bud-take when used for budding than did comparable healthy buds. A better take was recorded for virus-infected buds than for healthy buds of several sweet-orange varieties, Eureka lemon, Columbia sweet lemon, Dancy tangerine, and 2 varieties of West Indian limes. Healthy and diseased buds from seedlings of 6 grapefruit varieties, tested under similar conditions, showed no difference in bud-take.

It is not understood why infected grapefruit buds also did not give a better take than healthy ones. It was considered that this might be due to the fact that grapefruit plants have tissues nontolerant to tristeza, but this explanation is not applicable because West Indian limes, which also have nontolerant tissues, showed a definite difference in bud-take.

The better bud-take obtained with virus-carrying buds, even when budding was practiced on the nontolerant sour-orange rootstock, indicates that tristeza virus had no detrimental effect on the building of callus tissue at the bud union.

The tristeza virus apparently induces an accumulation of synthates in the infected scion budded on a nontolerant rootstock in the early phase of decline.[3] This suggests that the better take obtained with buds from diseased plants might have resulted from the presence of a higher amount of food material or hormones in infected bud tissues than in comparable healthy buds. The effect induced by the disease would probably correspond to the horticultural practice of girdling the branches of certain trees before budwood is taken, in order to insure better bud-take.

It is still to be determined whether buds from tristeza-infected symptomless carriers, such as sweet-orange on a tolerant rootstock or sweet-orange seedlings, will take better than virus-free buds from comparable sources.—Instituto Agronomico, Campinas, Brazil, and Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

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REPORT AND ABSTRACTS OF THE 1952 ANNUAL MEETING OF THE SOUTHERN DIVISION, AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division of The American Phytopathological Society has concluded another successful annual meeting, held in conjunction with the 49th annual convention of the Association of Southern Agricultural Workers in Atlanta, Georgia, on February 4, 5, and 6. A joint session was held with the Crops Division of the Agronomy Section. A total of 54 papers was presented. As a result concurrent sessions were held for the first time in the Southern Division. Approximately 130 plant pathologists were in attendance at the divisional sessions.

The following officers were elected at the business meeting on February 5: *President*, J. H. Jensen; *Vice-President*, A. L. Harrison. The offices of *Secretary-Treasurer* and *Councilor* are administered by J. A. Lyle and Coyt Wilson, respectively.

JAMES A. LYLE, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Stability of strains of sugarcane mosaic virus. ABBOTT, E. V. Strains A, B, and D of sugarcane mosaic virus have been carried as stock cultures for 15 years on the clones P.O.J. 234, Co. 281 and Louisiana Purple, on which they produce identical symptoms, without change in the characteristic symptoms produced when transferred to the clone C. P. 31/294, the differential host for these strains. Strain C, which produces characteristic symptoms as compared with the other 3 strains on all these clones, has also remained stable. Strains A, B, and C have been carried for more than a year on C. P. 31/294 without evident change in symptoms, but strain D after 3 to 6 mo. on this clone, shows a gradual change from the characteristic discrete, elongate, yellowish-white lesions, to the mild mottling typical of strain A. When juice from plants showing this symptom change was inoculated to healthy C. P. 31/294, only strain A symptoms were produced. This phenomenon has been observed by 3 workers in this Division working independently at different times with different accessions of strain D.

Rhizoctonia solani, a foliage pathogen of forage legumes and grasses in southeastern United States. ALLISON, J. LEWIS. Sclerotial isolates were made on PDA from blighted foliar parts of alfalfa, big and birdsfoot trefoil, Ladino clover, lespedeza, soybean, garden bean, tall fescue grass, orchard grass, bermuda grass, smooth brome grass, and bluegrass, representing collections from several localities in the southeastern states. Cultural types occurred among isolates; however, no consistent type was associated with a single host and similar types were obtained from different hosts. The optimum temperature for growth of all isolates was 25° to 30° C. Pathogenicity of isolates was determined on the host from which they were obtained and by cross inoculation to the other hosts listed. Free moisture on the foliage was necessary for infection and development of blight symptoms. Optimum temperature for disease development was 25° to 30° C. A range in virulence from high to low was obtained among isolates from a specific host and all isolates reacted in a like manner on the other hosts. Most isolates produced coarse mycelial growth on infected foliar parts although a few produced a silky threadlike mycelial growth. All isolates produced large sclerotia on certain hosts and a few produced micro-sclerotia on one host. Results indicated that several strains of *Rhizoctonia solani* are foliage pathogens of forage legumes and grasses in southeastern United States.

Fungicides for turnip-green leafspot control. ANDES, J. O. Leafspot caused by *Cercosporella albomaculans* is serious in some seasons, especially after the first cutting, due to a build-up of inoculum. Control sufficient for com-

mercial processing was obtained by 2 applications of either 3-3-50 bordeaux or 1 pound ferbam to 50 gal. water, but bordeaux caused severe injury. For the second crop a bordeaux application immediately after the first cutting had an eradicant action and the plants were not materially damaged at this stage. Ferbam applied approximately 10 days later protected the foliage until harvest with no objectionable residue. Fixed copper and zineb spray and dusts were unsuccessful, due apparently to insufficient adherence in a period of frequent showers.

Forage crop Rhizoctonia cross inoculation tests. ATKINS, J. G., JR. Various forage crop plants grown in south Louisiana are often severely damaged by Rhizoctonia aerial blight during summer months under humid conditions. Fields or experimental plantings of fescue, trefoil, and Lespedeza have been rather severely affected by the disease. Of the various weeds or wild grasses also attacked, Bermuda grass showed a general infection. Studies have shown that the fungus attacking the cosmopolitan Bermuda grass was the same as on fescue, trefoil and Lespedeza. The "sasakii type" of Rhizoctonia was consistently isolated from each. The aerial parts of various plants grown in steamed soil were inoculated and held for 48 hr. under conditions favorable for infection, high humidity and temperature. In cross inoculation tests, isolates from the 4 hosts were pathogenic on fescue, trefoil and Lespedeza as well as soybeans, snapbeans, cowpeas and fig. However, cowpeas showed some resistance. The results obtained indicate that the Rhizoctonia blight found in Louisiana on the rather recently introduced Alta and Kentucky 31 fescue and that observed on Bermuda grass for a number of years are caused by the same fungus.

Studies on inheritance of resistance to red rot disease of sugarcane. AZAB, Y. E., AND S. J. P. CHILTON. Studies were made for a period of 2 years on the reaction of progenies of 14 crosses of sugarcane to the red rot fungus *Physalospora tucumanensis*. The crosses involved resistant × resistant parents, resistant × susceptible parents, and susceptible × susceptible parents. The stalks were inoculated by a spore suspension of the fungus, then were buried. Two to 3 mo. later, they were dug up, split lengthwise and examined. The hypothesis is advanced that resistance in sugar-cane to red rot is governed by 1 or a few genes for resistance from *Saccharum spontaneum* plus a dominant inhibitor gene from *Saccharum officinarum*. The inhibitor gene from *S. officinarum* masks the effect of genes for resistance from *S. spontaneum*. A group of minor modifier factors also influences the degree of reaction of the plants to the disease. The irregular chromosomal behavior occurring in sugarcane varieties during meoisis influences the ratios of resistant to susceptible plants obtained in the progenies.

Transmission of mosaic resistance to progenies in crosses between certain sugarcane varieties. AZAB, Y. E., AND S. J. P. CHILTON. Studies on inheritance of resistance to sugarcane mosaic virus were made by inoculating 71,769 sugarcane seedlings grown from true seeds which came from 175 crosses between different varieties of sugarcane. The seedlings were inoculated in the greenhouse by juice obtained from plants of the sugarcane variety Co. 290 showing mosaic symptoms, using 220-mesh carborundum as an abrasive. Both resistant and susceptible plants were found in all types of crosses. The percentages of plants showing mosaic symptoms in the progeny of crosses involving resistant × resistant, susceptible × susceptible, and resistant × susceptible parents ranged from 0.0 to 87.7, 2.6 to 35.6 and 0.8 to 94.5 per cent, respectively. From the data obtained the hypothesis is advanced that inheritance of resistance to mosaic disease of sugarcane is conditioned by complementary factors, with resistance being dominant. A group of minor modifying factors affects the degree of expression of the disease. Irregularities in behavior of *Saccharum spontaneum* chromosomes during meiosis in sugarcane varieties also influences the transmission of resistance from the parents to their progenies.

Scirrhia acicola, a cause of loblolly pine needle browning. BOYCE, JOHN S., JR. *Scirrhia acicola* is known to cause the damaging brown spot needle blight of longleaf pine seedlings. Field examinations and collections in the Carolinas and Georgia in 1949 and 1950 indicated that it can also cause extensive needle dieback on loblolly pines. Current needles of pole-size and larger trees as well as of seedlings and saplings have shown severe browning in fall and winter. Proof of the pathogenicity of *S. acicola* to loblolly pine needles was obtained by spraying seedlings with water suspensions of conidia derived from a single-spore culture. The pines were kept in iceless refrigerators for several days after spraying. Inoculations were begun in February 1951 in the greenhouse and made periodically through August. Needle dieback, with the production of acervuli, was observed 2 to 5 mo. after inoculation on many needles that were at least ⅓ their ultimate length at the time of inoculation.

Factors to be integrated in control of Southern blight on peanuts. BOYLE, LYTTON W. When the experience of the farmer and results of tests to control Southern blight on peanuts are viewed as a whole, they appear more like the chance distribution of some factor as yet considered insignificant, than as true correlations. When reports on the nature of *Sclerotium rolfsii* were examined from this viewpoint, the following hypothesis was evolved. Reduce crop losses by denying the organism a medium for growth in the upper stratum of soil, where conditions are most favorable to its development. From the results of preliminary tests based on this hypothesis, it is obvious that control for this disease becomes an integration of 3 phases separated according to respective levels on the plant. Control of the disease on the root, on the lower stems, and at the soil line are accomplished independently. For example, the proper combination of land preparation with respective methods of cultivation has obtained infection on the stems of 81 per cent of a stand without root infections. Complete burial of weeds and crop refuse, without any fringe reaching above a 3-inch depth, reduced infection on roots. Preemergence compounds and flat culture have given encouraging results. Leaf spot control must also be a part of completely integrated control.

Wild Plum in relation to the control of phony disease. BRUER, H. L., AND C. E. SHEPARD. A survey by the acid test method for phony disease in wild plum, initiated in January 1950, is continuing. 18,000 tests from 600 locations in 83 counties of 11 states have been made. Phony in wild plum was found in Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee and Texas. Positive reactions have been secured from 4 species of native plum: *Prunus angustifolia, P. injucunda, P. Munsoniana,* and *P. umbellata.* Area of disease occurrence in wild plum roughly coincides with that of occurrence in peach but is not contingent upon proximity of diseased peach orchards. Zones of high, moderate, and low, disease incidence are also roughly coincident for the 2 hosts. Spread from plum to peach is frequent in zones of general disease occurrence. In zones having factors unfavorable for spread, phony may occur in plum without appreciable spread to peach. Infected plum serves as a reservoir of phony virus when diseased peach trees are removed. As a step in control of phony disease in peach orchards, wild plum should be eliminated from orchard environs. Plum should be eliminated in advance of peach planting for maximum results.

Control of peanut root knot by soil fumigation and by crop rotation. COOPER, W. E. Peanut root knot, caused by *Meloidogyne hapla* Chitwood, is widespread in the peanut growing area of North Carolina. The disease is very destructive in heavily infected soils. Row applications of Dowfume W-40, Dowfume N, D-D Mixture, or D-D Mixture

plus 15 per cent chloropicrin, each at approximately 1.5 and 3.0 ml./ft. of row controlled root knot and resulted in normal yields. In untreated check plots the yields were reduced approximately 70 per cent. At the higher rate of application all materials were equally effective. A rotation experiment was initiated in heavily infested soil in 1950. Using a split-block design Roanoke soybeans, corn, cotton and peanuts were planted in both fumigated (Dowfume W-40, 18 gal./A) and non-fumigated blocks. The entire area was planted to peanuts in 1951. The effect of crop rotation and soil fumigation on root knot control was evident in root knot indices and peanut yields in 1951. Preceding crops, other than peanuts, reduced root knot and increased yields in both fumigated and non-fumigated blocks. Within rotation treatments root knot indices were lower and yields were higher in the fumigated than in the non-fumigated blocks.

Recent studies on lupines in Florida. DECKER, PHARES, A. T. WALLACE, AND T. E. WEBB. Blue lupine, *Lupinus angustifolius*, released to the growers in Florida in 1939 and yellow lupine, *L. luteus*, released in 1945 have been successfully grown as winter legume cover crops. Non-alkaloid strains of both blue and yellow have been used for winter grazing and cover. The acreage of lupine increased rapidly until 1950-51 season when cold weather, insect and disease pests caused an estimated loss of 75 per cent of the total crop. The lupine improvement program based upon cultural practices, use of chemicals for disease and insect control, and breeding, through selection and testing, have failed to prevent increased losses in lupine production. A breeding program based upon crosses between the more promising commercial strains and the native and introduced species of lupines was initiated in 1949 in an effort to produce a vigorous, sweet lupine, adapted to the area, and resistant or tolerant to the disease and insect pests. Crosses between high and low alkaloid strains in both blue and yellow lupine have been made and the F2 generation are now growing in the field. The low alkaloid strains are usually low in vigor and tend to revert to high alkaloid when grown in the field. Many of the native species are resistant to certain disease and insect pests. These species are being used in the breeding program. Treating air dried seed with X-rays at 500 to 30,000 r. units at 80,000 volts has produced mutations that may be of agronomic value.

Crown rot of strawberry. FELIX, E. L. Strawberry crown and root rot, apparently caused by the unidentified fungus reported by Sherbakoff in 1924 has been observed further in Tennessee during the past 5 years. Symptoms consist of colorless to brownish rot of roots and subterranean stems, frequently covered with a conspicuous white mold, especially just beneath the mulch or soil surface, often extending into the surrounding areas. The lower portion of the stems may be girdled or completely rotten, and mealy; the upper affected only in the outer tissues with rotting and sloughing of lower petioles. Stands appear unreduced, even in rows 100 per cent infected. Suckers invariably become infected from diseased plants; runners do not. Incidence is throughout the year, equally on all varieties tested. A 1949 isolate produces similar symptoms on seedlings of *Fragaria virginiana*, grown aseptically. It is characterized by the presence of clamp connections, coarse mycelial strands consisting of fasicled hyphae; occasional concentric zonation and sclerotical formation; production of decided acidity; narrow pH range and high optimum temperature for growth and, so far, sterility. Approximate pH relations (buffered solutions): min. 2.2-3.3, opt. 4.5 and max. 5.3, suggesting possible control at pH 6.0-6.5; temperature (unbuffered potato-dextrose agar) min. 11.5, opt. 27-32 and max. 35° C.

Rhizoctonia blight of strawberry. FELIX, E. L. Severe blight, caused by *Rhizoctonia solani*, resulted in death of strawberry plants in localized spots in experimental plots at Knoxville, Tennessee, during the past 3 years. Leaflet infection was entirely independent of soil contact, and root or fruit infection. The disease first appeared on the leaflets as large brown areas, which soon involved the entire leaf blades, petioles, and finally the whole plant. Sclerotia were not observed in the field, but formed on infected leaves placed in a moist chamber. A white to grayish superficial mold on petiole bases of other strawberry plants in 1951 proved to be the Corticium stage of the fungus, although no experimental connection between the forms or blight was attempted. Rhizoctonia blight was observed only occasionally on certain crosses and has not been observed thus far on commercial varieties of strawberries.

Reaction of cowpea varieties to Fusarium wilt. HARE, W. W. Seventy-eight cowpea varieties or strains were tested against the 2 races of Fusarium wilt defined by Armstrong. Plants grown in seed flats were inoculated 3 wk. after planting by removing, dipping roots in a suspension of the organism, and re-setting. Final notes were taken about 5 wk. after inoculation using living (resistant) and dead (susceptible) classifications. Wilt reaction, and also agronomic characters, varied greatly, frequently in seed stocks with the same name. With varietal resistance and susceptibility defined as insufficient susceptible plants for a 3:1, and too many for a 1:3 ratio, respectively, and all others intermediate, varieties were grouped as follows: susceptible to both races—all Brown Sugar Crowders, a Cream Crowder, and a catjang; resistant to *1*, susceptible to *2*—8 Blackeyes, 2 Chinese Reds, a Cream Crowder, Holstein, Potomac, Rice, and Sumptious; susceptible to *1*, resistant to *2*—Groit; and resistant to both—Alabrowneye, Arlington, two Brabhams, Calhoun Crowder, Iron, Six Weeks Ala, Speckle, and 4 unnamed strains. Of the remaining 45, 8 were intermediate to *1*, susceptible to *2;* 10 intermediate to both; 25 resistant to *1*, intermediate to *2;* and 2 intermediate to *1*, resistant to *2*.

Terminology in phytopathology. HILDEBRAND, E. M. A revamped or expanded terminology is needed to define in particular two happenings, the 1951 freeze-out of citrus in the Rio Grande Valley and death of over 1 million shade trees in Houston, Texas, during the drouthy summer of 1951.

While the terminology in phytopathogenic disorders is well-defined and accepted, confusing terminology has been employed for non-pathogenic diseases. Fortunately, improper use of "physiological" has been dropped, but supplanting this by "physiogenic" leaves much to be desired. Physiogenic means inadequate nutrition due to deficiency, excess, or unbalance in mineral nutrients which, when corrected, makes the plant normal or healthy. Injuriogenic refers to mechanical damage or environmental injury. Environmental excesses of cold, heat, rain, drouth, light, dark, fumes, etc., can be controlled only in enclosures. Climatic diseases are found especially in subtropical areas due to the frequent introductions of temperate-zone plants that often suffer and die from insufficient dormancy, etc. Plants out of climate and out of photoperiod lose vitality and die prematurely. Such weakening is frequently accompanied by pathogenic and secondary invaders. The term "Climatic Disease" is suggested for such non-parasitic disorders causing plant failure for which other designations do not apply.

The association of the sting nematode with some persistent cotton wilt spots in northeastern South Carolina. HOLDEMAN, Q. L., AND T. W. GRAHAM. Root examinations in 8 fields of wilted cotton of a resistant variety failed to yield evidence of nematodes. However, washings from soil samples revealed the presence of the free-living sting nematode, *Belonoliamus gracilis*. In a preliminary experiment infested field soil was collected and divided into 2 lots: one lot was air-dried; the other was kept moist. The separate soil lots were potted and seeded to cotton. After 58 days nematode populations of each pot were examined. Air-drying had eliminated the sting nematode from the soil. Cotton Fusarium inoculum was added to each pot. The 2 soil lots were subdivided and reseeded with resistant

and susceptible cottons. In the presence of the sting nematode the resistant variety averaged 52 per cent wilt; the susceptible variety averaged 88 per cent wilt after approximately 10 wk. In the absence of this nematode the resistant variety showed no wilt; the susceptible variety averaged 10 per cent wilt. A second collection of field soil gave similar results. Although other parasitic nematodes were present in all pots the loss of wilt resistance was correlated with high sting nematode populations.

A lethal (virus) disease of fluecured tobacco, new to the old belt. JENKINS, WILBERT A. Characteristic symptoms are produced externally and internally on diseased plants of strictly agronomic type. On leaves immature at infection, definite but faint mosaic patterns are present, unaccompanied by puckering of the tissues. Midveins and frequently other prominent veins are blackened, the necrosis generally continuing through the petiole into the cortex of the stem to cause sunken necrotic streaks throughout its length. Older leaves exhibit necrotic lesions while very young leaves and flower buds are blackened. Internally, necrosis of the cambium prevails within the stem and into the roots, while the pith develops extensive patches of black concentric rings. The symptomatology is reminiscent of the reaction of *Nicotiana glutinosa* to TMV, except for leaf mottle and failure of the growing points to become warped and twisted. Preliminary inoculations, using field material, indicate the presence of more than a single virus. Necrosis and/or mottle develops within 10 to 14 days depending on the method of inoculation, confirming field observations that the disease was introduced with topping and suckering operations. The identity of the viruses is not known but 3 possibilities are being considered: 1) Plantago virus, 2) a TMV strain that causes necrosis in N'N' varieties, and 3) a virus complex.

Some factors influencing the behavior of disease-resistant fluecured tobaccos during the 1951 growing season. JENKINS, WILBERT A. A number of rather well-defined biotic complications such as root knot, root rot disease complexes, soreshin, etc., have long been recognized as factors which contribute to erratic behavior of disease-resistant tobaccos, principally because they suppress vegetativeness or provide more favorable infection courts for the lethal parasites causing black shank and Granville wilt. Less well-defined climatic influences have also been considered. During the past field season, limited field areas afforded an excellent opportunity for evaluation of a number of these factors, individually and collectively. In a number of locations extreme losses from black shank or wilt could be attributed to root knot or root rot disease complexes. In other locations extreme losses were sustained on rotated land. Under such conditions, losses were conditioned by 1) failure of rotation to control biotic factors, 2) partial drowning of the crop, 3) high humidity (temporary) and temperature which conditioned attack by pathogens high above the soil level, thus encountering relatively susceptible tissues. Drouth, while generally present, was not a dominant factor except in the presence of one or more of the other factors.

The effects of a series of seed protectants on repression of fungi in and on corn seed and their effect on growth of seedling corn. JOHNSON, J. RALPH. This experiment, conducted in 1951, (1) tested the effectiveness of 5 seed protectants; 4 per cent M.E.M.A., 6.70 per cent Agrox, 3.35 per cent Mergamma, 1.00 per cent Semesan Jr. and Arasan on seed corn; and (2) compared the reactions of 4 varieties and hybrids, namely, Golden Cross Bantam, Hastings Prolific, Dixie 18, and N.C. 27 to these protectants. Laboratory tests in petri dishes were used to determine effects of the above protectants on germination of seed, tap root development, and suppression of seed borne fungi. The Semesan Jr. gave outstanding results in suppression of fungi. The tap root development of the Arasan treated seed was farther advanced than the other protectants. A greenhouse experiment was also conducted to determine the effects of the protectants on stand, growth, weight and color of plants. On the basis of the rating of the plants in pots as to emergence, growth, and color, Semesan Jr. was first followed closely by 6.70 per cent Agrox, and 3.25 per cent Mergamma. The first group was significantly better than Arasan and M.E.M.A. The rating of the varieties showed Dixie 18 first followed respectively by Hastings Prolific, N.C. 27, and Golden Cross Bantam. During an enforced dry period Dixie 18 showed more resistance to drought than the other varieties and hybrids. All treated corns were far superior to the untreated corns in this phase of the experiment. All seed treatments proved beneficial in the laboratory and greenhouse experiments and gave significantly better results in terms of growth, color, and emergence than the checks.

The effect of actinomycetes antibiotic to Phythium Arrhenomanes in plate cultures on root rot of sugarcane and corn. JOHNSON, LEANDER F., AND H. E. WHEELER. Several hundred actinomycetes, isolated from Louisiana sugarcane soils, have been tested for antibiotic activity to *Pythium arrhenomanes*, which causes root rot of sugarcane and corn. The zones of inhibition produced when the actinomycete isolates were placed on one side of a petri plate containing Czapek's medium and a disc of *Pythium* placed on the opposite side varied from 0 to 40 millimeters. Plate cultures of 37 selected antibiotic isolates were mixed with autoclaved soil in clay pots and the soil was then infested with *Pythium arrhenomanes*. One wk. later corn was planted and the roots were examined after 2 wk. of growth. The best control of root rot was obtained with actinomycete isolates which produced a wide (30 mm. or more) zone of inhibition in petri plates. However, some such isolates did not give significant control of Pythium root rot. Certain isolates which produced a moderate (10 to 25 mm.) zone of inhibition in culture gave significant control, whereas other isolates which produced this moderate zone were not effective in controlling the disease. None of the isolates which produced a zone of inhibition of 10 mm. or less gave significant control of Pythium root rot.

Evaluation of resistance to Pseudomonas solanacearum in tobacco under greenhouse conditions. KELMAN, ARTHUR. Critical evaluation of Granville wilt resistance in tobacco under greenhouse conditions was obtained when inoculations with virulent isolates of *Pseudomonas solanacearum* were made by the following procedures: (1) puncturing the stem through a drop of bacterial suspension placed at a leaf axil and (2) cutting roots on one side and pouring a bacterial suspension over them. Stem inoculation of wilt-resistant Oxford 26 plants resulted in higher disease readings than root inoculations, whereas with a susceptible variety, 402, there were no real differences between disease indices of root and stem inoculations. Greatest differentiation between Oxford 26 and 402 was obtained by the root inoculation technique. Results were similar when the resistant Dixie Bright varieties, 27, 101 and 102, were compared with 402. Significant differences in disease rattings between the Dixie Bright varieties were also obtained, D. B. 102 being the most resistant, and D. B. 27 the least resistant. There was a highly significant negative correlation between increase in height following inoculation and disease index. This correlation indicates the potential value of height measurements in supplementing disease readings for the evaluation of Granville wilt resistance in tobacco.

Evaluation of techniques for inoculating corn with Physoderma maydis. KELMAN, ARTHUR, AND T. T. HEBERT. The effect of different inoculation procedures on development of brown spot of corn was studied in connection with field evaluation of corn lines for resistance. Inoculations were made in the whorl using (1) dry sporangia, (2) 10 ml. of a sporangial suspension in water and (3) 10 ml. of a suspension of germinating sporangia. In 1950 and 1951 the most

uniformly high disease readings were obtained with sporangial suspensions. Inoculation with dry sporangia was most effective when rains followed inoculation whereas inoculation with suspensions of germinating sporangia was most effective when there were no rains in the week following inoculation. Inoculation when plants were approximately 3 ft. tall was more effective than inoculation when plants were 4 to 6 ft. tall. Although relatively high disease ratings were obtained with only 1 inoculation, an increase in brown spot severity was obtained by 1 or 2 additional inoculations at 10-day intervals. Resistant, intermediate and susceptible lines were inoculated with suspensions of approximately 500, 5,000 and 50,000 sporangia per ml. The highest concentration of sporangia gave the highest infection ratings on all lines, but best differentiation between resistant and susceptible lines was obtained with the concentration of 5,000 sporangia per ml.

Comparison of a phloroglucinol test with the acid one for phony. KENKNIGHT, GLENN. The following freshly prepared mixture of methyl alcoholic solutions in the ratio 10:1:1 is about equal in speed to 30 per cent HCl in methyl alcohol as a developer of the phony reaction and has the advantage of containing much less acid: $ZnCl_2$ saturated at 20°C., 2 per cent phloroglucinol, and 20 per cent HCl. The phloroglucinol test solution gives more apparently positive reactions than does the acid test solution. On wood cross-sections, the phloroglucinol solution frequently develops red dots in the wood, indicative of a positive reaction, up to a few cm. beyond the point where the wood ceases to react to the acid solution. On tangential wood sections an apparently positive test is indicated by red streaks in which, under magnification, the globes of gum plugging the vessels resemble chains of red beads. On such sections the phloroglucinol solution develops more striking color reactions and gives more apparently positive reactions than does the acid solution. The phloroglucinol test appears to be less specific for phony than does the acid test, because on several occasions apparently positive reactions were obtained on tangential wood sections of peach and plum (*Prunus angustifolia*) affected with peach rosette when such reactions were not obtained with the acid test.

Germination inhibiter in wood and bark of peach and wild Prunus. KENKNIGHT, GLENN. Seeds of squash, cantaloupe, sweetcorn, cabbage, turnip, blue lupine, and chokecherry on damp filter paper over wet peach-wood or peach-bark sawdust in Petri dishes either completely failed to germinate or germinated after several days' delay, whereas in parallel controls (without sawdust) the seeds germinated well. Filtered water extracts of 1 gm. of peach-wood or peach-bark sawdust in 10 cc. of tap water used to wet filter paper for germinating seed also reduced and retarded germination. The inhibiting character of the extract persisted after dilution with water, but the degree of dilution inhibiting germination varied considerably from one experiment to another. It was correlated somewhat with seed size, being greatest with small seeds. The greatest dilution causing inhibition recorded was with alfalfa seed. An extract of 1 gm. of peach-root-bark sawdust (fresh weight), soaked 1 hr. in 10 cc. of water, then brought to a boil, filtered through paper, and diluted 50 to 1, retarded and reduced alfalfa seed germination. The germination-inhibiting substance appeared to be present in stem and root wood and bark of both apparently normal and phony (virus) peach and in root wood and bark of *Prunus angustifolia* and *P. serotina.*

Fungi associated with soybean seeds within the pods at Stoneville, Mississippi, in 1951. KILPATRICK, R. A. Pods of 10 varieties and strains of soybeans, representing 5 different maturity groups, were picked at weekly intervals before and after maturity. Seeds were removed from the pods under aseptic conditions and plated on potato-dextrose-agar containing the sodium salt of 2,4-D acid (50 mg. per L.). The purple-seed-stain fungus, *Cercosporina kikuchii,* comprised more than 50 per cent of the total isolates, although purple discoloration of the seed was observed only infrequently in the field. Other fungi isolated were: *Fusarium oxysporum, Alternaria* spp., *Phomopsis sojae, Aspergillus* sp., *Phyllosticta* sp., *F. roseum, F. solani, Trichoderma* sp., *Curvularia* sp., *Penicillium* sp., and several unidentified. *C. kikuchii* was isolated more frequently from the seed of late-maturing varieties than from early-maturing varieties. The reverse was true of *Phomopsis sojae. C. kikuchii* was isolated from the seeds before visible appearance of purple discoloration on the seeds and before symptoms developed on the external portion of the pods. Green seeds yielded relatively few fungi while mature seeds with a lower moisture content were from 75 to 100 per cent infected with fungi. Flower remnants, insect injuries, natural cracking of the wall, and systemic infection are possible means of entry into the pods.

Survival of the purple seed stain fungus in soybean seeds. LEHMAN, S. G. Purple stain of soybean seeds is caused by the fungus *Cercosporina kikuchii.* Germination tests with infected seeds showed that in some lots the purple stain fungus died in a high percentage of seeds between the first and second planting season after harvest. In other lots the fungus was still alive in many seeds 3 months before the second planting season. The fungus had died in all seeds previous to the third planting season. Infected purple stained seeds were adjusted to moisture levels of 6.3, 10.8, and 15.3 per cent. Seeds of each moisture level were stored 10 mo. in closed jars at temperatures of 8, 12, 16, 20, 24, and 28° C. The fungus lost viability more rapidly in seeds of high and medium moisture than in seeds of low moisture. Loss of viability by the fungus in seeds of high and medium moisture was markedly accelerated by storage at 16 to 20° C. or above. Only slight loss of viability of the fungus occurred at 8 or 12° C. regardless of moisture content of the seeds. At the higher temperature and moisture levels the seeds lost viability less rapidly than the fungus in the seeds.

Results of dusting tests for reduction of fungus damage to seed cotton. LEHMAN, S. G. An attempt was made at 2 locations in North Carolina to reduce boll rot and tight lock damage caused by fungus invasion of cotton bolls in early stages of opening. Tribasic copper sulphate, Dithane Z-78 (zineb), and Orthocide 406 (N-trichloromethylthio tetrahydrophthalimide) were used. These fungicides were mixed with toxaphene, CP-5 dust sticker and pyrophyllite diluent. Check plots received only toxaphene. Applications were made at weekly intervals through the boll opening period. At one location where insects had been well controlled previous to initiation of the test, the percentage of bolls damaged by fungi alone or insects and fungi was very low. Here the mean percentage of open bolls damaged was 8.3 on check plots, 12 on copper plots, and 7.3 on Dithane plots, the differences being insignificant. At the second location insect control was poor. Here the percentage of the opened bolls damaged by fungi alone was low, ranging from 3.1 on Orthocide plots to 5.6 on Dithane plots, the check and copper plots being intermediate. However, a high percentage of the bolls showed fungus damage associated with insect injury. These percentages ranged from 39 on Orthocide plots to 42 on no-fungicide control plots. Neither tribasic copper, Dithane Z-78, nor Orthocide 406 significantly reduced the percentage of bolls damaged by fungi alone or by fungi entering through injuries by insects.

Black shank control in tobacco plant beds. LUCAS, G. B., AND E. L. MOORE. Concurrent with the spread of black shank in the field the disease has become prevalent in tobacco plant beds. Since 1947 tests have been conducted in North Carolina to find a chemical soil treatment which would control black shank in the plant bed. Seventeen different materials were tested at different rates. Cyanamid, Uramon plus Cyanamid and fertilizer formula 16-6-2, widely used as plant bed weedicides, did not control black shank. Methyl bromide (15 per cent by volume in Xylene) and

chloropicrin, applied as drenches at rates of 255 and 38 ml. per sq. yd. respectively, in 1 gal. of water and methyl bromide gas at the rate of 1 lb. per 10 sq. yd. controlled the disease. Methyl bromide gas killed the black shank fungus buried 6 in. deep in sandy loam soil. Laboratory and greenhouse tests strongly indicate that Dixie Bright 101 and Dixie Bright 102 had more resistance in the seedling and transplant stage than Oxford 1 and Oxford 3. Dixie Bright 101 had as much resistance as Dixie Bright 102.

Fungi antagonistic to pythium arrhenomanes isolated from Louisiana sugarcane soils. LUKE, HERBERT H. A survey of Louisiana sugarcane soils to determine the occurrence of fungi antagonistic to *Pythium arrhenomanes*, the causal agent of sugarcane root rot, was intiated in December 1950. A total of 46 soil samples, from 13 different localities, were studied. The number of fungi per gram of soil ranged from 90,000 to 400,000; the average being 151,539. Approximately 4,000 fungal isolates were tested to determine their ability to inhibit the growth of *Pythium arrhenomanes* in artificial culture. Of these 15.36 per cent were found to be antagonistic; *Penicillium spp.* comprised 44.16 per cent, *Aspergillus spp.* 22.61 per cent, *Spicaria spp.* 22.40 per cent, and other fungi 10.83 per cent. Zones of inhibition produced by the organisms varied from 1 to 20 mm.; 82 per cent produced a zone of inhibition of less than 10 mm., 17 per cent produced a zone of inhibition ranging from 11-15 mm., while only 1 per cent produced a zone of inhibition larger than 15 mm. These preliminary studies have shown that certain soil fungi are antagonistic to *Pythium arrhenomanes;* moreover, further studies will be necessary to determine the role played by these antagonistic soil fungi in the development of sugarcane root rot.

The presence of internal mycelium in corn grains in relation to external symptoms of corn ear rot. MILLER, JULIAN H. Previous surveys of corn ear rots in Georgia have shown approximately 95 per cent *Fusarium moniliforme*, 3 per cent *Diplodia zeae*, 1 per cent *Gibberella zeae* and 1 per cent of others such as species of *Penicillium*, *Aspergillus*, and *Rhizopus*. In all cases grains from ears judged disease-free have given rise to *F. moniliforme* after surface sterilization in cultures. This has not been true of the other fungi. In a recent similar study of ears from breeding stocks from the Tifton Experiment Station 64.4 per cent showed *D. zeae*, 12.2 per cent *D. frumenti*, 9.6 per cent *D. macrospora*, 9.6 per cent *F. moniliforme* and 3 per cent *Helminthosporium carbonum*. The ears varied from ones with no visible symptoms to definitely rotted. *D. zeae* in this study was now the chief pathogen even in apparently disease-free grains. In a study of sections the mycelium appeared to grow from the cob at the butt end through the grain attachment, some filling the cavities between the embryo and the scutellum and between the coleoptile and the plumule and immediately under the coleorhiza. Embryonic tissues were the first attacked, and starchy and horny endosperm and pericarp disintegrated only in the most advanced stages. *D. macrospora* produced similar symptoms. When *F. frumenti* and *H. carbonum* were present visible symptoms appeared immediately and all tissues seemed equally susceptible.

Host-parasite relations of Phytophthora parasitica var. nicotianae in roots of resistant and susceptible tobacco varieties. NUSBAUM, C. J. Cytological studies of black shank infections in roots of susceptible (402) and resistant (Dixie Bright 101 and Dixie Bright 102) tobacco varieties were made following inoculation with water suspensions of zoospores of *Phytophthora parasitica* var. *nicotianae*. Zoospore germination and direct penetration of the epidermis occurs within 3 hr. In young roots of the susceptible var. the mycelium rapidly invades cortical tissues, both inter- and intra-cellularly, reaching the stele within 6 hr. Initially the robust mycelium ramifies through living cells, the walls and protoplasts of which are not visibly altered. Impoverishment of these cells follows, resulting in depletion of their contents. In older lesions, all tissues, including the xylem, are invaded. Tyloses are formed in xylem elements. In roots of resistant varieties, epidermal penetration is similar to that in susceptible plants. The epidermal cell, entered by the infection hypha, and adjacent cells collapse. The mycelium develops feebly in the tissues killed in advance of the invading hyphae. In most cases, infection is limited to the cortex. If infection reaches the stele, however, tissue invasion is similar to that in the xylem and pith of susceptible varieties.

Estimating the inoculum potential of Phytophthora parasitica var. nicotianae in the soil. NUSBAUM, C. J., G. B. LUCAS AND J. F. CHAPLIN. In field evaluations of tobacco breeding stocks for resistance to black shank, critical levels of soil infestation of the causal organism, *Phytophthora parasitica* var. *nicotianae*, are essential in order to eliminate susceptible plants from segregating populations. Observations in North Carolina indicate that the infestation level may vary greatly from field to field or even in different parts of the same field. Information is lacking regarding the persistence of the fungus in the soil and the factors that may either increase or reduce the inoculum potential. Seeking an approach to these problems, the need of a technique for the quantitative estimation of inoculum potential has been recognized. Various laboratory and greenhouse methods have been explored, involving the growing of susceptible plants in various dilutions of infested soil with steamed sand and the application of standardized soil samples and soil washings to fresh tobacco stem cuttings. Preliminary soil dilution tests have shown that certain naturally infested soils, diluted 1 to 100 or even higher with steamed sand, contain sufficient inoculum to infect and kill susceptible plants within a 20-day period. The top dilution in which there is no plant survival within a 20-day period and the rate of death at this dilution are used to compute the inoculum potential.

Pathogenicity of an undescribed Sclerotium sp. OWEN, JOHN H. A fungus producing purple sclerotia and pathogenic on watermelon was isolated in Florida and compares favorably with a fungus isolated about the same time from decaying tobacco stalks by Wolf in North Carolina. On potato-dextrose agar, the fungus produces a fast-growing white, fluffy mycelium with the formation of soft, bright purple sclerotia which range from ovoid to flat and irregular in shape, are produced singly or in clusters, and measure 1 to 4 mm. in diameter. In the young stage the sclerotia are light purple, becoming darker as they mature (Ridgeway, Color Standards and Nomenclature, Mauve and Madder violet). The septate mycelium remains white during all stages of growth, and clamp connections are very common in the young hyphae. It has a pH range of 2.0 to 8.8 with an optimum of 6.0 and cardinal temperatures of 13°, 30°, and 37°C. Inoculation experiments have shown it to be pathogenic on seedlings of lupine, cucumber, tobacco and peanut.

Phony Peach Disease—A Review of Organized Control from 1929 to 1951 and the Effect of Recent Developments on Future Control Programs. PERSONS, T. D. This is a report of surveys made by the control project in 26 states during the past 20 years, progress in regulating nursery stock, and results of commercial orchard control in severely affected areas. Phony infected peach trees were found in 17 states. Infections in most states were light and subsequent inspections indicated that the disease had been eliminated by control procedures from 6 states and from 175 of the 295 affected counties in 8 others. Resurveys have shown that the disease has reappeared in a number of counties where it had apparently been eradicated. This suggested reinfection from wild hosts, and wild plum surveys in such areas seem to demonstrate definitely that this host is responsible. A review of nursery regulations from Federal Quarantine No. 67 to the present uniform state quarantines is presented. Present quarantine requires nursery site approval based on location at least 300 yd. from wild plum,

½ mile from infected commercial orchards, and ½ mile from urban areas. Control in commercial areas is effective if diseased tree roguing is practiced annually on an area-wide basis. Combination of annual inspection and removal of diseased trees, wild plum elimination, and vector control gives promise of further reducing disease incidence in commercial areas.

Progress report on internal cork of sweetpotatoes. RANKIN, HARVEY W. Internal cork of sweetpotatoes is a virus disease transmitted by grafting and aphids. The principal commercial variety, Rorto Rico, is extremely susceptible. Other varieties and selections have been observed as being less susceptible. In 1950, 164 varieties and selections were tested for resistance to internal cork, using grafting and aphid technics for transmission. All but 22 of these selections were eliminated in these tests. These selections were tested again in 1951. Most of them showed a high degree of resistance and a few selections have shown no symptoms of Internal cork whatever. These varieties and selections are available for breeding work. Efforts to find a reagent that would give a reaction indicating the internal cork virus in plant material have been negative. Experiments using antibiotics and chemicals in an effort to inactivate the internal cork virus in diseased plants have given negative results. Manipulations of soil pH for the purpose of finding out if pH influenced the expression of internal cork symptoms have shown that it does not. Octa-methylpyrophosphoramide has shown the greatest promise as a spray material for the control of insect vectors of this disease.

Cereal root-rot studies in Tennessee. REED, H. E. Wheat yields are low despite good fertility; barley is often severely stunted and rotted at the crown, hence the role of root pathogens as a causal factor is being investigated. Of the numerous organisms isolated from roots of wheat, barley, oats, and rye, only *Helminthosporium sativum* and *Pythium arrhenomanes* were found pathogenic. *H. sativum* was the more important root rot organism on barley causing losses from 40 to 70 per cent. Such practices as too early fall seedling while the weather is warm, and barley following barley or other gramineous crops have been directly correlated with disease occurrence and severity. Field tests with 150 local barley lines showed little or no resistance to either spot blotch or crown root rot. Inoculation tests with other barley lines in search of root rot resistance has indicated possibilities. Late fall seeding, rotation with non-gramineous crops, and seed treatment were shown to minimize the disease.

Seasonal relations of silky thread blight, Rhizoctonia ramicola W. & R. ROBERTS, D. A. AND GEORGE F. WEBER. Silky thread blight of certain perennial ornamental plants in Florida caused by the vegetative state of the fungus was published in 1951. Up to that time observations in nature failed to reveal sclerotia or spores. Cultural variations applied to the fungus in the laboratory for the purpose of producing sclerotia or spores were unsuccessful. The fungus on about 50 per cent of the previously known diseased plants failed to survive the 1950-51 winter, which began with a frost in late November and continued below average for most of the time. On some plants during the summer of 1951 it appeared in typical fashion, or even more destructive than previously observed. It failed to develop on certain crepe myrtle, holly, coral bean and guava plants that were diseased during the previous season. Additional plants of most of these species showed the disease, however, with high bush blueberry, *Vaccinium irrgatum* Ait., as a new host. The disease was favored by the wettest summer, with the highest temperatures, for many years. The fine silky hyphae were abundantly produced but lacked sclerotia which are the conspicuous, diagnostic signs of other aerial *Rhizoctonia spp.* During August the basidiomycetous stage of the fungus was collected on several host plants and further study is in progress.

Eradication and thinning tests for Nectria and Strumella canker control in Maryland. ROTH, ELMER R. In a heavily cankered mixed oak stand in western Maryland, Nectria- and Strumella-cankered trees were felled on 11 acres 4 times between 1933 and 1948. Observations up to 1951 showed very little increase in Nectria cankers either on the treated or the adjacent check area. Most of the Nectria cankers formed during the first 25 years and thus prior to establishment of the experiment. There was a 40 per cent increase in Strumella cankers on the treated area and a 22 per cent increase on the check, in 1951 over 1933. Thus, felling of heavily cankered, pole-sized oak in Maryland was unnecessary for Nectria and ineffective against Strumella. Since *Urnula craterium* has recently been regarded as the perfect stage of the Strumella and since it fruited abundantly on down oak, the ineffectiveness and even possible harmfulness of felling cankered trees can be understood. Thinning failed to accelerate the rate of healing of Nectria or Strumella cankers over the 16 yr. period, and dominant trees healed cankers proportionately no faster than suppressed trees.

The Incidence of Phony Disease in Plum Thickets as Related to Proximity of Infected Peach Orchards. SHEPARD, C. E., AND H. L. BRUER. Surveys of wild plum by the chemical testing procedure demonstrate widespread infection with phony disease. Disease incidence, roughly indicated by percentage of stems tested reacting positively, varies widely among individual thickets. Investigations were made during 1951 in 4 central Georgia counties to determine whether this variation is correlated with proximity to infected peach orchards. Approximately 40 stems were tested from each of 69 thickets with prescribed spatial relationship to infected peach orchards. The following results were obtained. Phony disease in plum was found not to be dependent on peach as a source of infection. No appreciable difference in incidence of infection could be demonstrated between plum thickets immediately adjacent to infected peach orchards and those located at varying, relatively small, distances from such orchards. Plum thickets in an area densely populated with infected peach had a much higher average incidence of infection than thickets in an area widely removed from commercial peach planting but otherwise of similar ecology. Individual thickets varied too widely for any prediction of incidence based on this difference. Thickets in an uncultivated area, surrounded by pine woods, had an average incidence least of all; but again, certain individual thickets had a very high incidence of infection.

The inheritance of resistance to Fusarium wilt in upland and Sea Island cotton. SMITH, ALBERT L. AND J. B. DICK. Studies were made in naturally infested soils on the inheritance of resistance to *Fusarium oxysporum* f. *vasinfectum* using Cook 307 (resistant) and Hurley's Rowden (susceptible) as parental material of upland cotton, *Gossypium hirsutum*. Resistance in *G. barbadense* was studied with combinations of Seabrook, Sea Island (resistant) and Hurley's Rowden. Resistance in upland cotton to Fusarium wilt is apparently determined by 1 dominant factor. However, nematodes in the soil provide the primary portal of entry for the wilt organism. Therefore, resistance in cotton to nematodes considerably affects the severity of the wilt infection and the reaction of the host to wilt. In an effort to eliminate partially the effect of the nematodes, the soil was treated with a soil fumigant. When the F_1, F_2, and F_3 generations were grown on treated soil, ratios obtained indicated 1 dominant factor for Fusarium resistance. When the F_1 generation from the cross Seabrook × Rowden was grown on untreated soil, all plants were resistant. On treated soil the F_2 segregation indicated 2 dominant factors for Fusarium resistance. Results obtained in the segregating generations from the cross Cook × Rowden grown on untreated soil indicated that multiple factors were involved. These results further emphasize that nematodes condition the reaction of the host to Fusarium wilt. By transferring

the resistance in *G. barbadense* to upland cotton by the backcross method, selecting for nematode resistance following each backcross, a high degree of resistance to Fusarium wilt should be obtained.

Some north Georgia apple production problems. TAYLOR, JACK. Spray tests conducted and observations recorded in north Georgia apple orchards during 1951 indicate that apple black rot (*Physalospora obtusa*) causes more loss to the grower than all other diseases and insects combined. Usually the fruit is affected as it begins to ripen. Soon after the symptoms appear the fruits drop prematurely. For this reason all drops were checked and scored according to the cause of damage. About 62 per cent of all drops was affected with black rot. About 73 per cent of Golden Delicious, 60 per cent of Red delicious, and 30 per cent of Stayman Winesap drops showed this disease. The difference between varieties was about the same in every orchard, and no difference could be attributed either to spray schedules or materials used. Records on Golden Delicious trees in Union County showed that more than 75 per cent of the fruit set on 32 trees dropped before harvest time. About 73 per cent of those drops was infected with the black rot fungus. Leaf symptoms caused by this organism were more severe in orchards sprayed with Bordeaux mixture than in those sprayed with ferbam. The unusually dry 1951 growing season in north Georgia inhibited extensive development of apple scab and bitter rot, but apparently had no effect on black rot.

Breeding tobacco for immunity to black shank. VALLEAU, W. D. Resistance to black shank in present resistant varieties of *N. tabacum* is insufficient. Such varieties are satisfactory only in a rotation, but *N. longiflora* is immune. The immune factor is completely dominant in the $F_1 \times T$ and $T \times L$. The first backcross with *N. tabacum* is sometimes completely immune. The second backcross produces a few immune *tabacum*-like plants. By using a backcross parent resistant to mosaic, black root rot, wildfire, and fusarium wilt each generation, there should be no difficulty in combining resistance to 5 diseases if the black shank factor can eventually be stabilized in a *tabacum* chromosome, as has been done with the wildfire resistance factor from *N. longiflora* and the mosaic resistance factor from *N. glutinosa*. Elimination of susceptible plants from a backcross can be accomplished by inoculating seedlings a few days old or inoculating vigorous young transplants with swarm spores of *Phytophthora parasitica* var. *nicotianae*. Transplants developing leaf spots in about 48 hr. can be eliminated. Slow-growing susceptible plants that escape can be eliminated in 48 to 72 hr. if a leaf of each is placed in distilled water containing swarm spores. Susceptible leaves start decaying in 40 to 60 hr., while immune leaves are unaffected. By this method, breeding for immunity can probably be conducted without the aid of a contaminated field.

Preliminary report on some nutritional requirements of Chalara quercina. WHITE, IRENE AND FREDERICK T. WOLF. Some of the nutritional requirements of *Chalara quercina* Henry, which causes oak wilt, have been determined in a preliminary way. In a synthetic medium containing dextrose as the carbon source, ammonium sulphate as the source of nitrogen, and McIlvaine's citric acid-Na_2HPO_4 buffers, the optimum pH for growth was found to be approximately 4.0. The optimum and maximum temperatures for growth are 20-23°C. and 30°C., respectively. *C. quercina* can utilize ammonium sulphate and a variety of amino acids as sources of nitrogen, but cannot utilize nitrate nitrogen. Aspartic acid, glutamic acid, arginine, asparagine, proline, alanine or ornithine were superior to other amino acids tested as sole sources of nitrogen. The level of free amino acids tolerated by *C. quercina* is rather low, and there are indications that cystine and perhaps other amino acids are quite toxic to the organism. Of 22 carbohydrates tested, only *D*-arabinose and rhamnose were not utilized. Dextrose, levulose, sucrose, and cellobiose were the best of the carbon sources tested.

A seedling inoculation technique for testing cotton varieties for resistance to Verticillium wilt. WILES, A. B. The seedling inoculation technique used in determining resistance to *Fusarium* wilt and *Verticillium* wilt in other crops has been adapted for studying resistance of cotton varieties to *Verticillium albo-atrum*. Seed flats filled with sandy loam soil were fumigated with methyl bromide prior to planting. In the 4-leaf stage the seedlings were inoculated by removing them from the soil and dipping the roots in a suspension of the wilt organism. The inoculum was prepared by growing the fungus in Czapek's solution, then passing it through a Waring Blendor prior to inoculation. Sufficient plain agar was added to give a thick consistency. Air and soil temperatures and soil moisture were maintained at levels adequate for host development. Seven to 8 days after inoculation wilting of the plants occurred, followed by yellowing and shedding of leaves. A brown discoloration of vascular tissues was observed in affected plants and the causal organism was isolated from such plants. Many seedlings died within 4 wk. following the treatment; other plants having wilt symptoms remained alive but were severely stunted. Varieties or strains of cotton with slight or no disease symptoms were considered resistant or tolerant. Comparable results with *Fusarium* wilt of cotton have also been obtained using this technique.

CHLORANIL, A COINED NAME FOR THE FUNGICIDAL CHEMICAL TETRACHLORO-PARA-BENZOQUINONE

The Special Committee on Fungicide Nomenclature of the American Phytopathological Society, cooperating with the Interdepartmental Committee on Pest Control, announces the selection and approval of the following common coined name:

Chloranil for the pure chemical tetrachloro-para-benzoquinone.

This name has been in use for a number of years as a designation for the pure chemical and has appeared in text books. This is now a common name for this fungicidal chemical, to be used in the same way as the previously selected names ferbam, ziram, nabam, zineb, and thiram. Approval of the name was requested by Dr. Dwight Powell, Chairman of the Special Committee from 1947 to December 1951.

Information concerning the name and the chemical has been filed with the Trade-Mark Division of the U. S. Patent Office, Department of Commerce, to preempt the use of the name as a trademark. Adoption of the name by the Special Committee on Fungicide Nomenclature of the Society carries with it the acceptability of the name for use in PHYTOPATHOLOGY. Approval of the name by the Interdepartmental Committee on Pest Control renders the name available for free use in designating the pure chemical tetrachloro-para-benzoquinone.—GEORGE A. ZENTMYER, Chairman, Committee on Fungicidal Nomenclature, American Phytopathological Society.